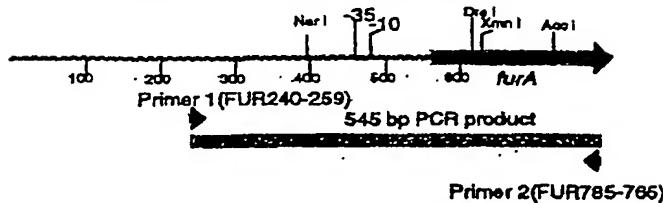


FIGURE 1A

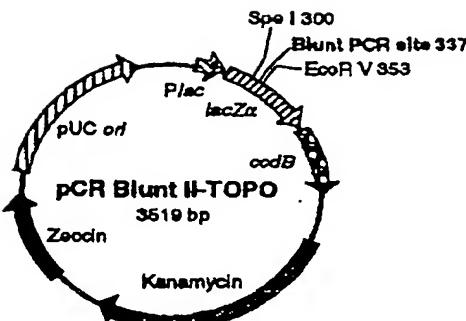
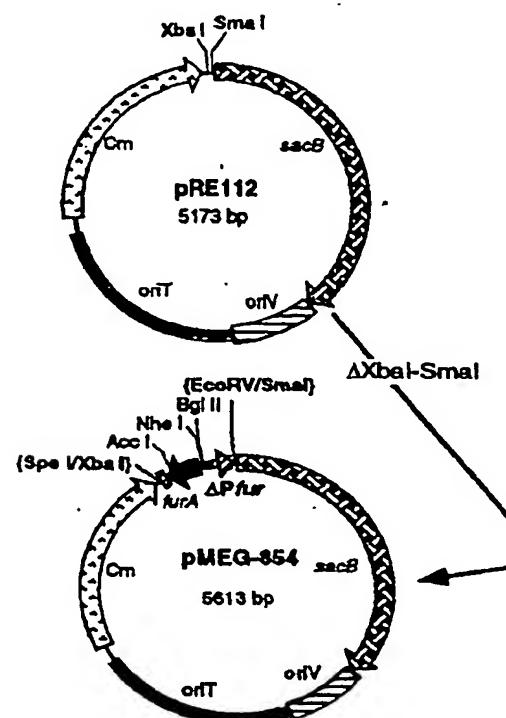
*S. typhimurium* chromosomal DNA

Primer 1: FUR240-259  
5'-TTT CGG CTG AAT TGC ACC TC-3'

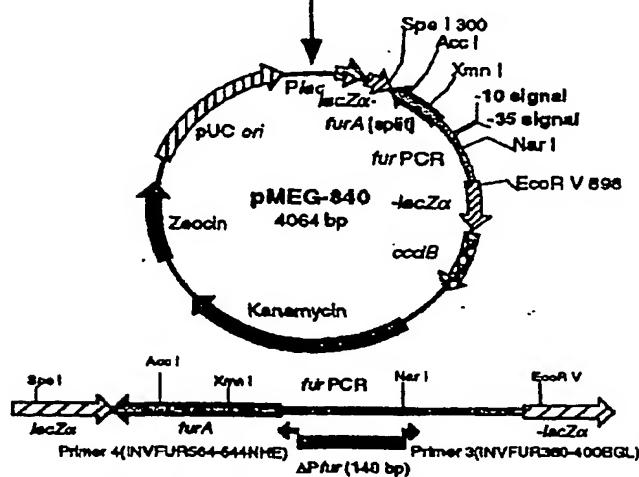
Primer 2: FUR785-765  
5'-CCT TCA AAA TTA TGG CGG GTC-3'

Primer 3: INVFUR380-400BGL  
5'-GAA GAT CTC CAC CTG ATT ACA CCC GGAC-3'

Primer 4: INVFUR564-544NHE  
5'-CGG CTA GCA ACA GGA CAG ATT CCG CATG-3'



Blunt ligation at the blunt PCR site of pCR Blunt II-TOPO vector



Inverse PCR to delete 140 bp P<sub>fur</sub> area

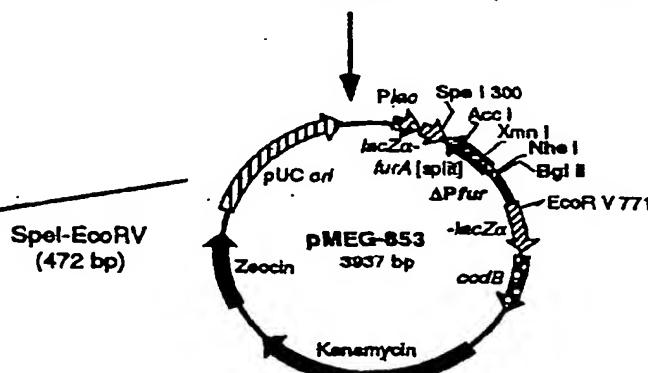


Figure 1-A. Construction of suicide vector for transfer of  $\Delta$ P<sub>fur223</sub>::TTaraC PBAD fur deletion-insertion mutation.

FIGURE 1-B

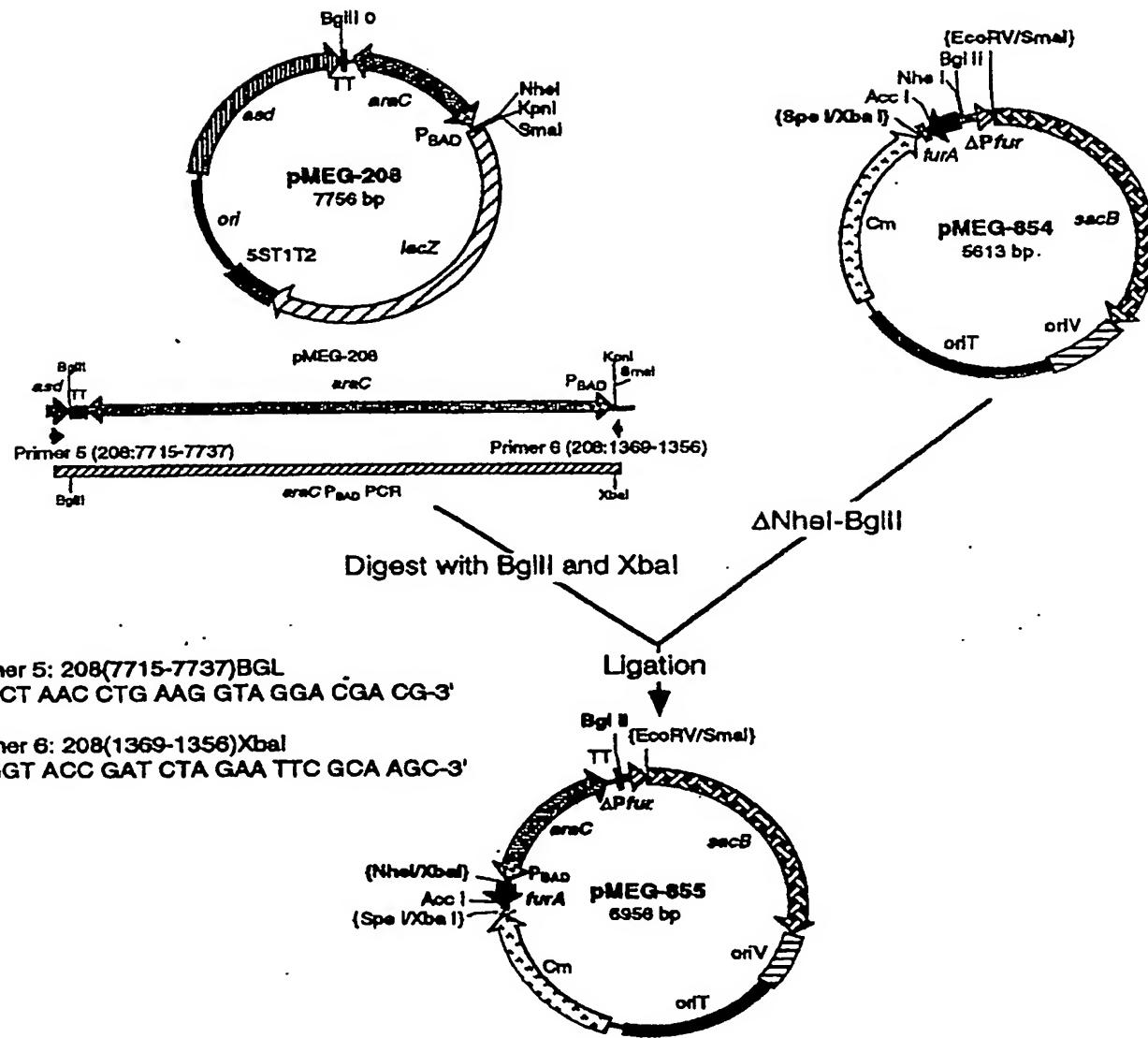
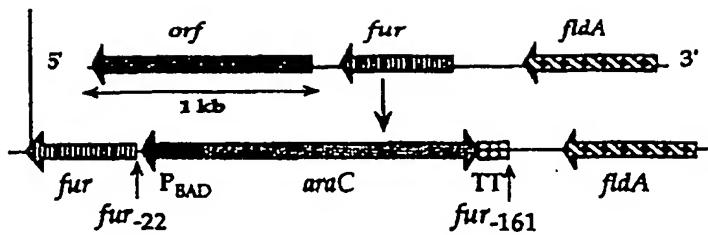


Figure 1-B. Construction of suicide vector for transfer of  $\Delta$ Pfur223::TTaraC PBAD fur deletion-insertion mutation.



140 bp *fur* promotor region deleted  
1,354 bp TTaraC P<sub>BAD</sub> inserted

Figure 2.  $\Delta$ Pfur223::TTaraC P<sub>BAD</sub>*fur* deletion-insertion chromosomal construction.

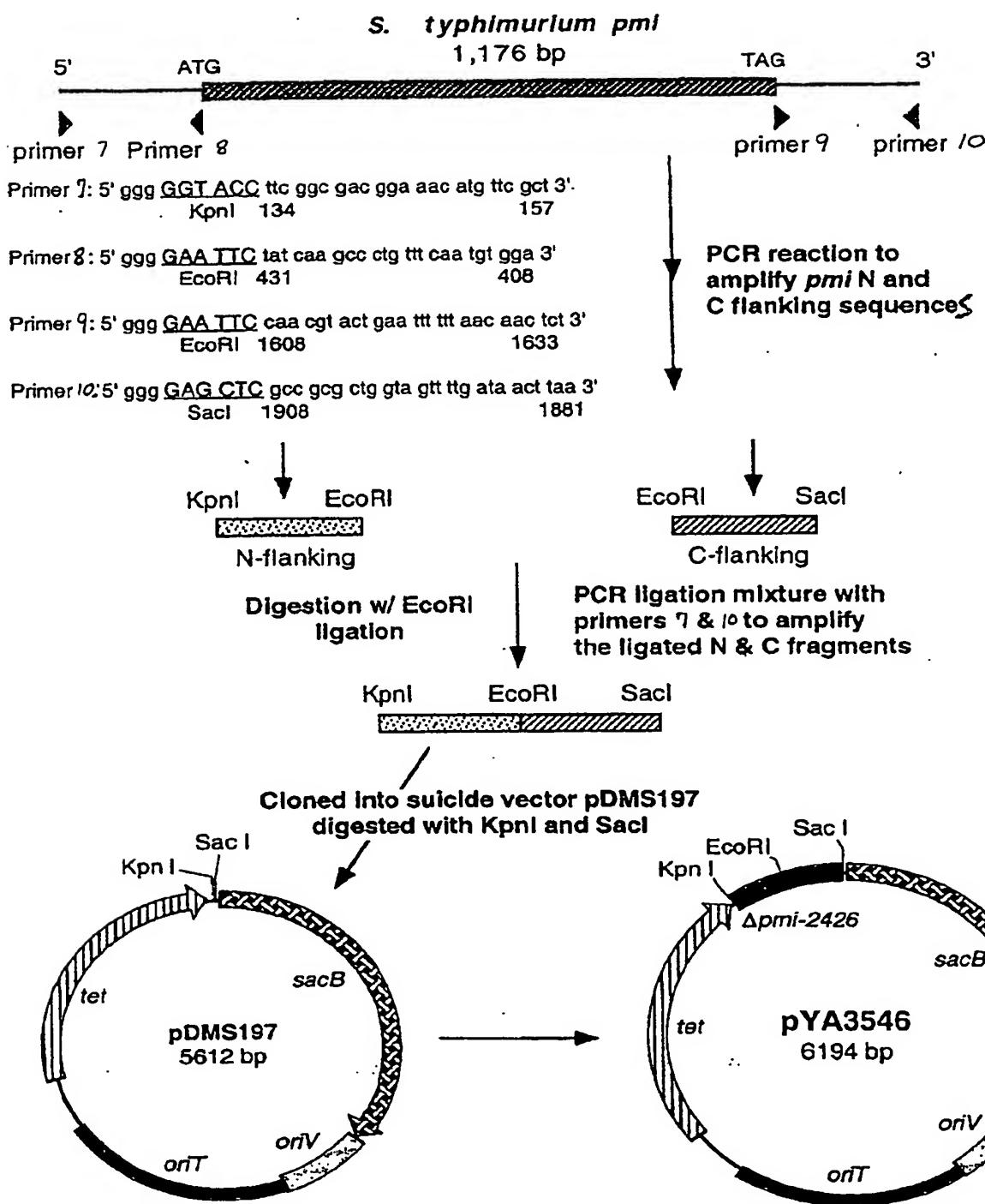


Figure 3. Construction of a suicide vector for *pmi* deletion,

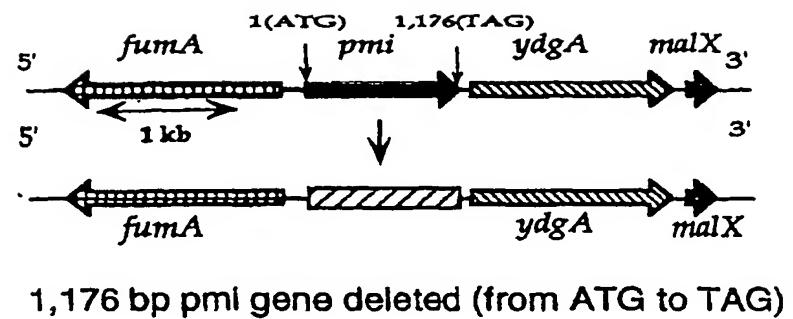
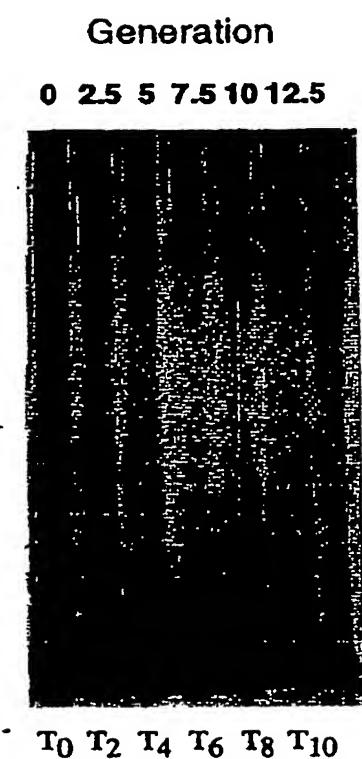
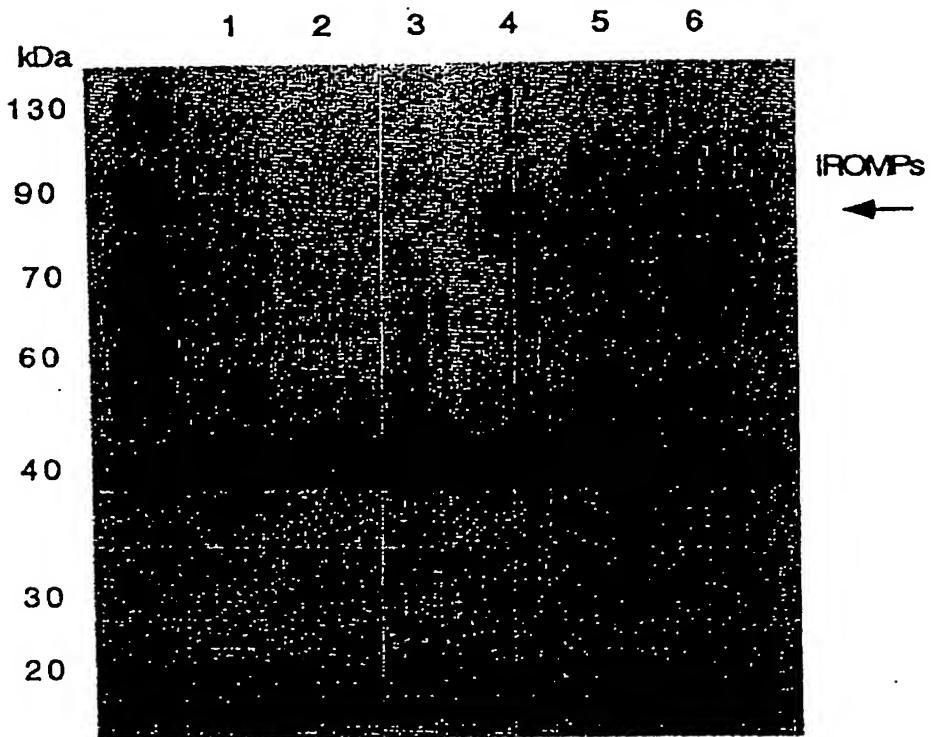


Figure 4. Chromosomal deletion for  $\Delta pmi$ -2426



**Figure 5. Reduction of LPS O-side chains in  $\chi$ 8650 as a function of numbers of generations of growth or times (hours) of sampling.**



Lanes:

1. $\chi$ 3761	wild-type	Nutrient broth (NB) + 0.2% arabinose
2. $\chi$ 3761	wild-type	NB
3. $\chi$ 8634	$\Delta$ Pfur223::TT araC P <sub>BAD</sub> fur	NB + 0.2% arabinose
4. $\chi$ 8634	NB	
5. $\chi$ 8754	$\Delta$ Pfur223::TT araC P <sub>BAD</sub> fur	$\Delta$ pmi-2426
		NB + 0.2% arabinose
6. $\chi$ 8754	NB	

Figure 6. Outer membrane protein profile of  $\Delta$ Pfur223::TT araC P<sub>BAD</sub> fur mutants grown in Nutrient broth +/- arabinose.

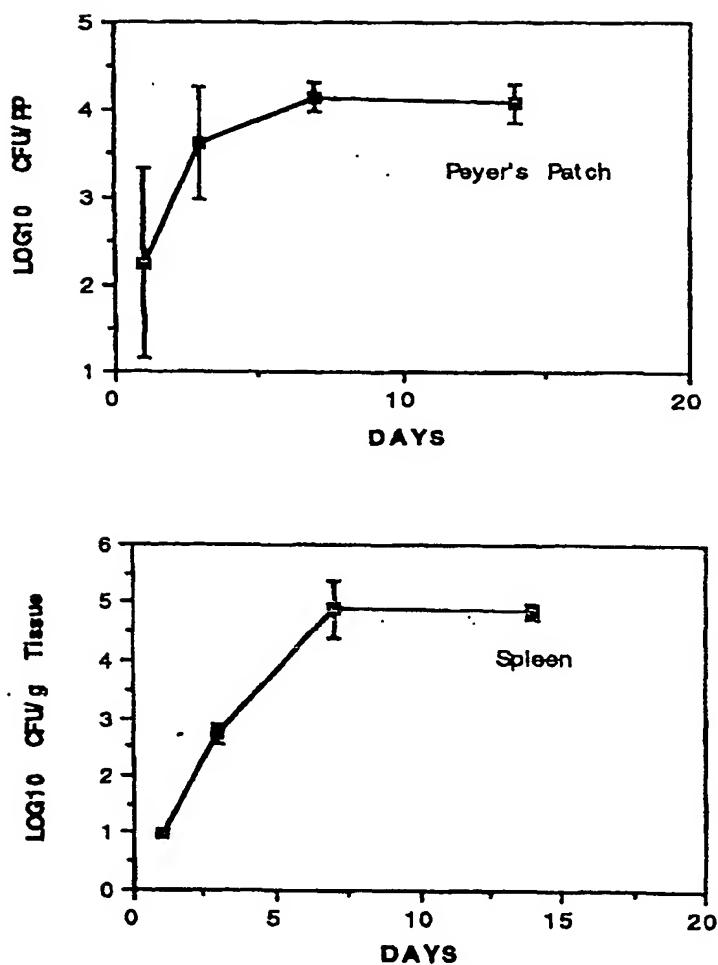


Figure 7. Colonization of 8-week-old female BALB/c mice with  $\chi$ 8634  $\Delta$ Pfur223::TTaraC P<sub>BAD</sub> fur following oral inoculation.

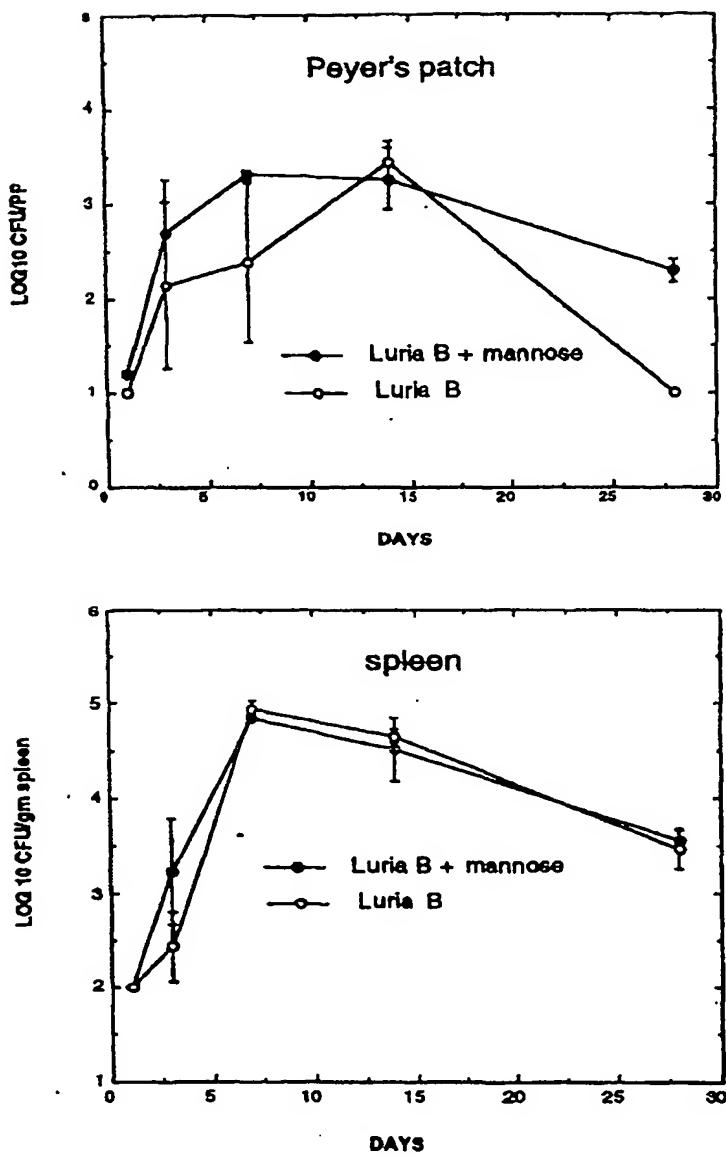


Figure 8. Colonization of 8-week-old female BALB/c mice with  $\chi$ 8650 ( $\Delta pmi$ -2426) following oral inoculation.

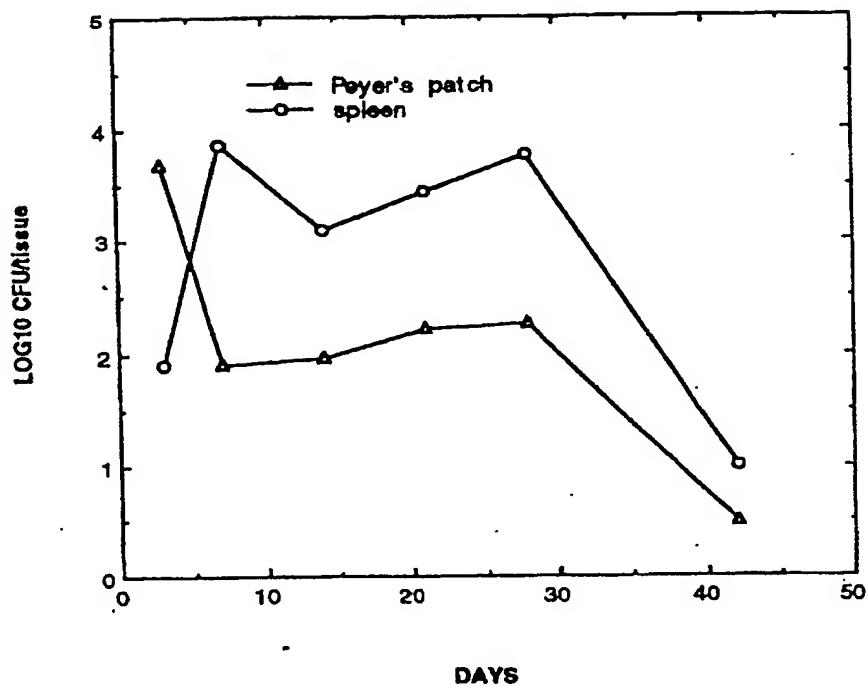


Figure 9. Colonization of 8-week-old female BALB/c mice with  $\chi$ 8754 ( $\Delta pml-2426 \Delta Pfur223::araC PBAD fur$ ) following oral inoculation.

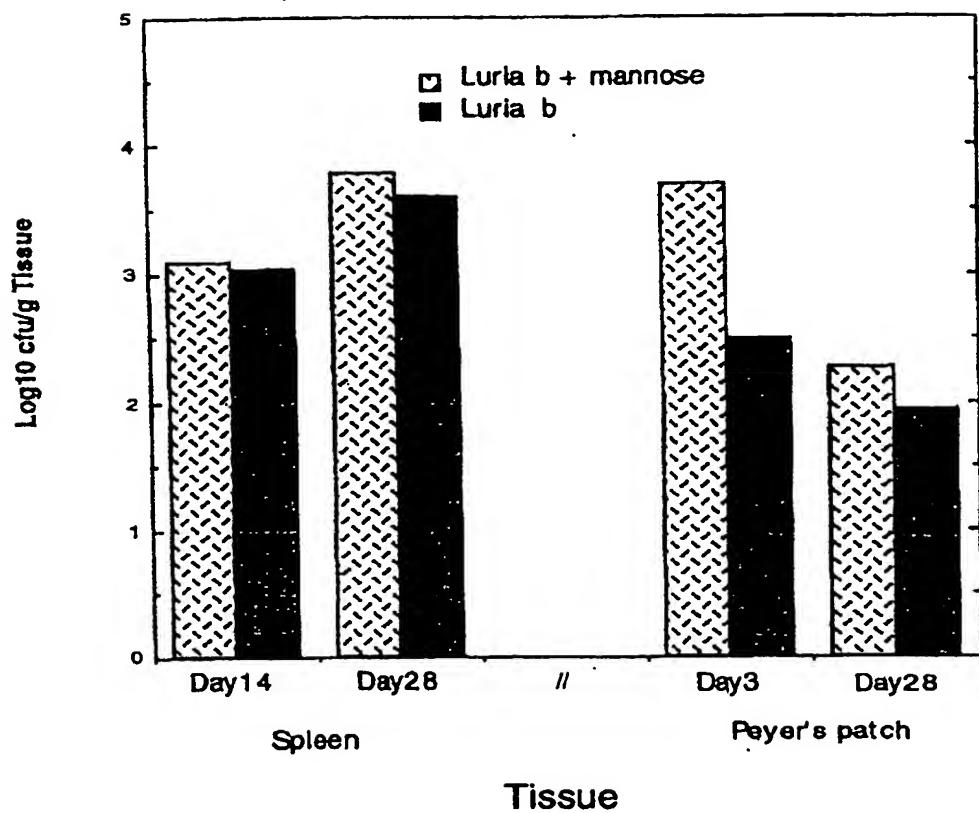
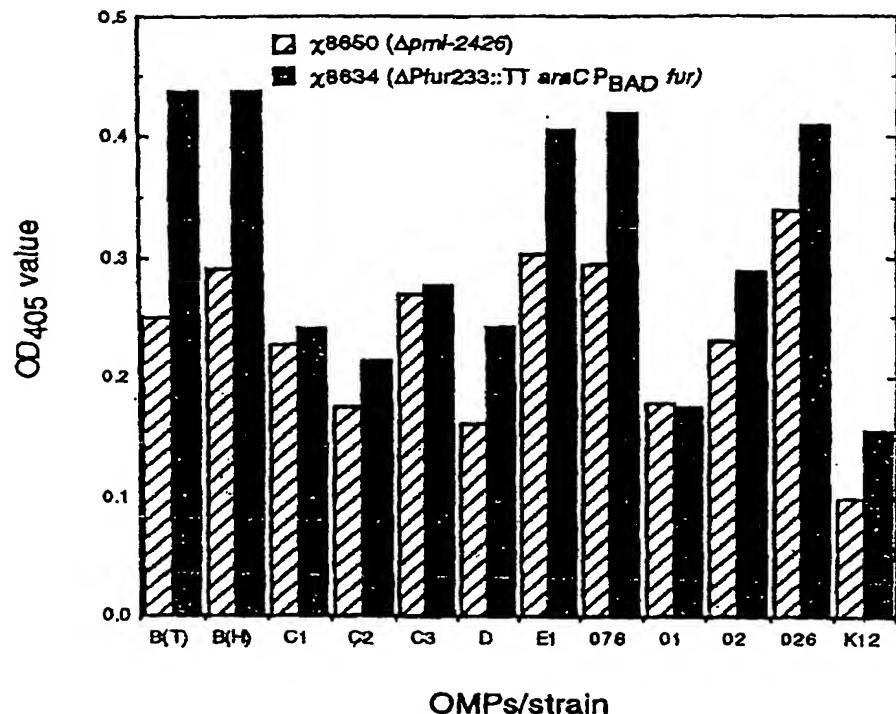
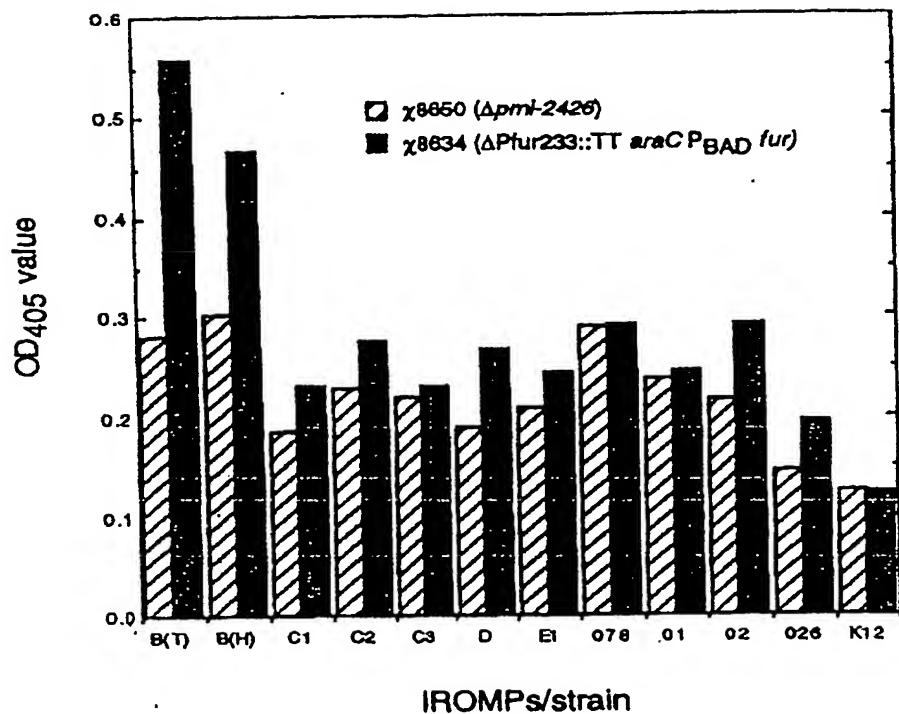


Figure 10. Colonization of 8-week-old female BALB/c mice with  $\chi 8754$  ( $\Delta pml-2426 \Delta Pfur223::TTaraC PBAD fur$ ) following oral inoculation.



B (T):	<i>S. typhimurium</i>	χ3761	E1:	<i>S. anatum</i>	χ4449
B (H):	<i>S. heidelberg</i>	χ3242	078:	APEC	χ7122
C1:	<i>S. infantis</i>	χ3212	01:	APEC	χ7237
C2:	<i>S. hadar</i>	χ3210	02:	APEC	χ7255
C3:	<i>S. albany</i>	χ3202	026:	EPEC	χ6206
D:	<i>S. enteritidis</i>	χ3700	K-12	<i>E. coli</i> K-12	χ289

Figure 11. IgG Ab responses to OMPs isolated from *Salmonella* and *E. coli* strains.



B (T):	<i>S. typhimurium</i>	χ3761	E1:	<i>S. anatum</i>	χ4449
B (H):	<i>S. heidelberg</i>	χ3242	078:	APEC	χ7122
C1:	<i>S. infantis</i>	χ3212	01:	APEC	χ7237
C2:	<i>S. hadar</i>	χ3210	02:	APEC	χ7255
C3:	<i>S. albany</i>	χ3202	028:	EPEC	χ6206
D:	<i>S. enteritidis</i>	χ3700	K-12	<i>E. coli</i> K-12	χ289

Figure 12. IgG Ab responses to IROMPs isolated from *Salmonella* and *E. coli* strains.

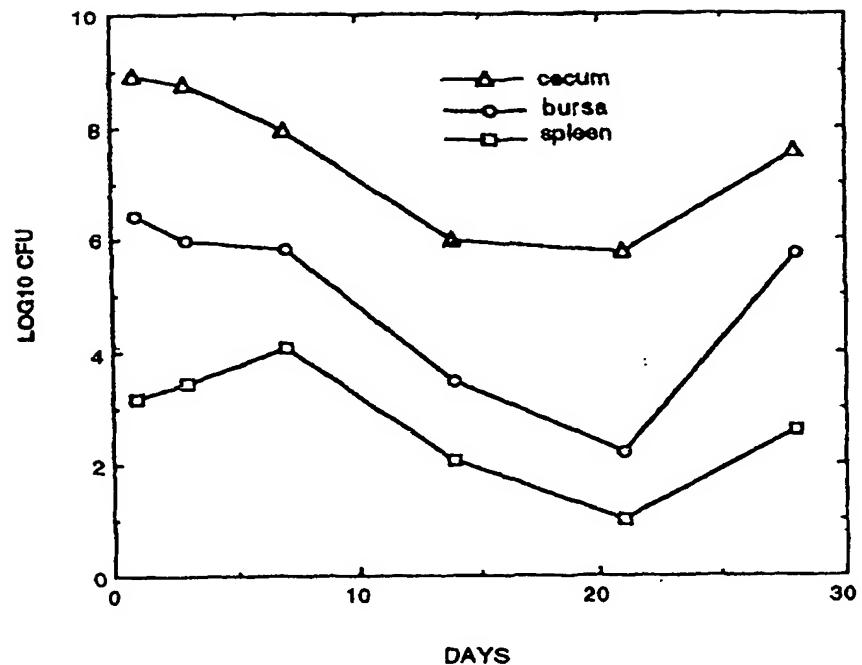


Figure 13. Colonization of day-of-hatch chicks with  $\chi$ 8754 ( $\Delta$ pmi-2426  $\Delta$ Pfur223::TT araC PBAD fur) following oral inoculation.

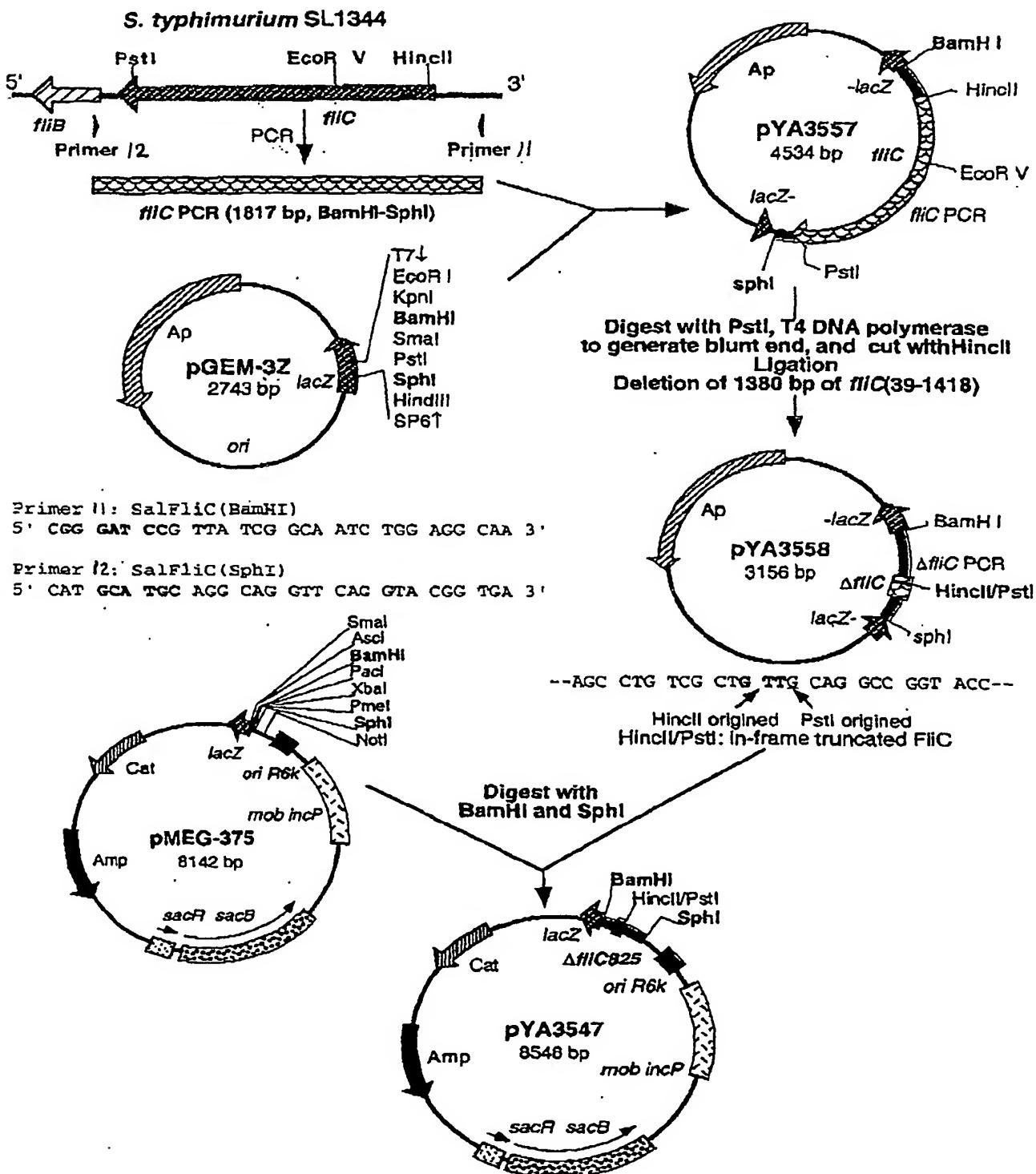
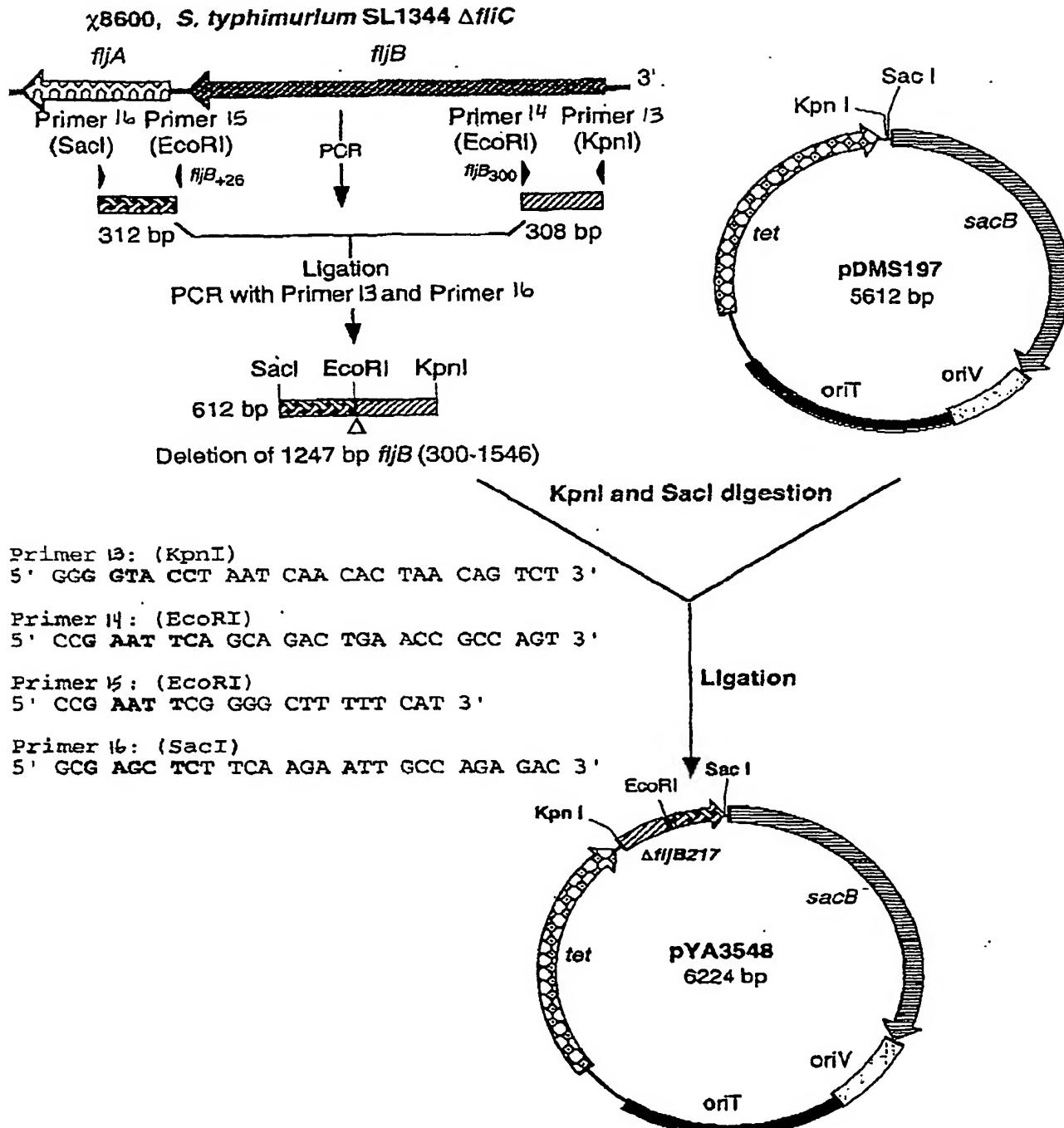
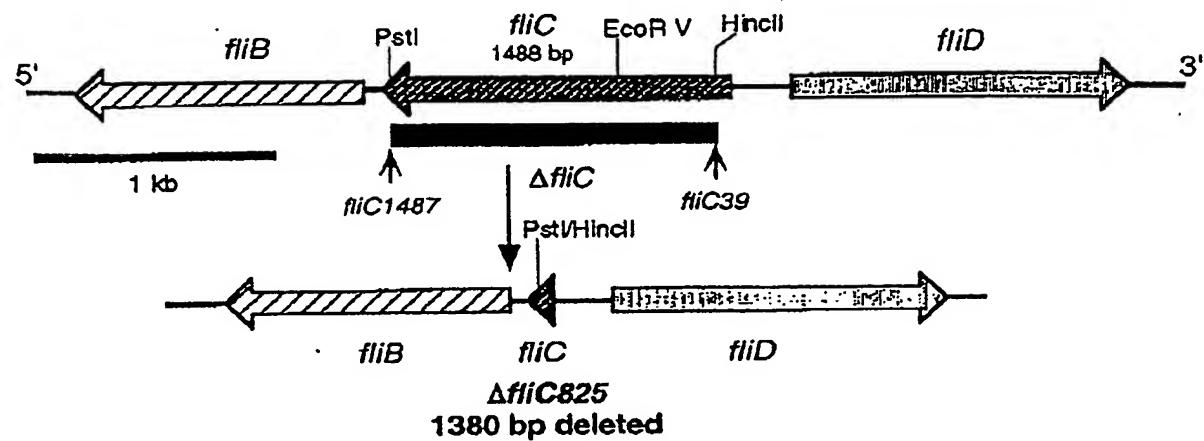
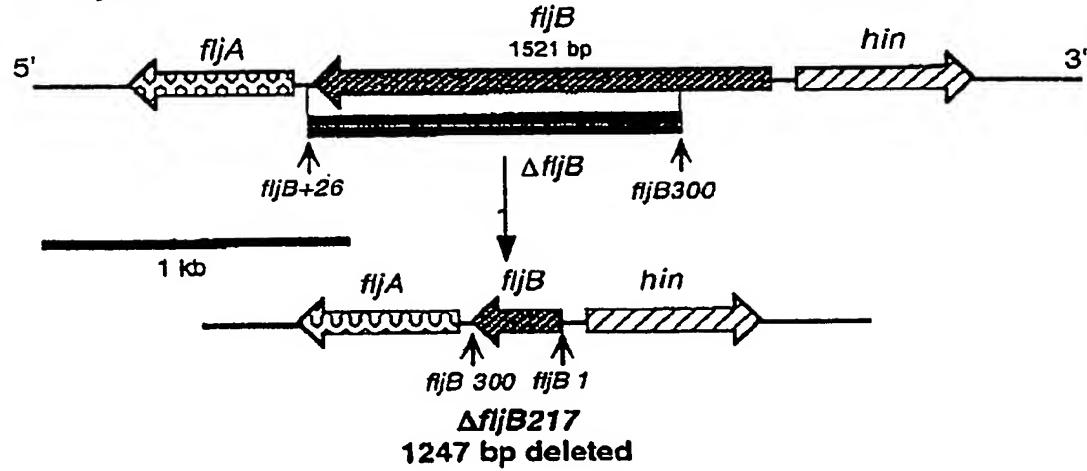
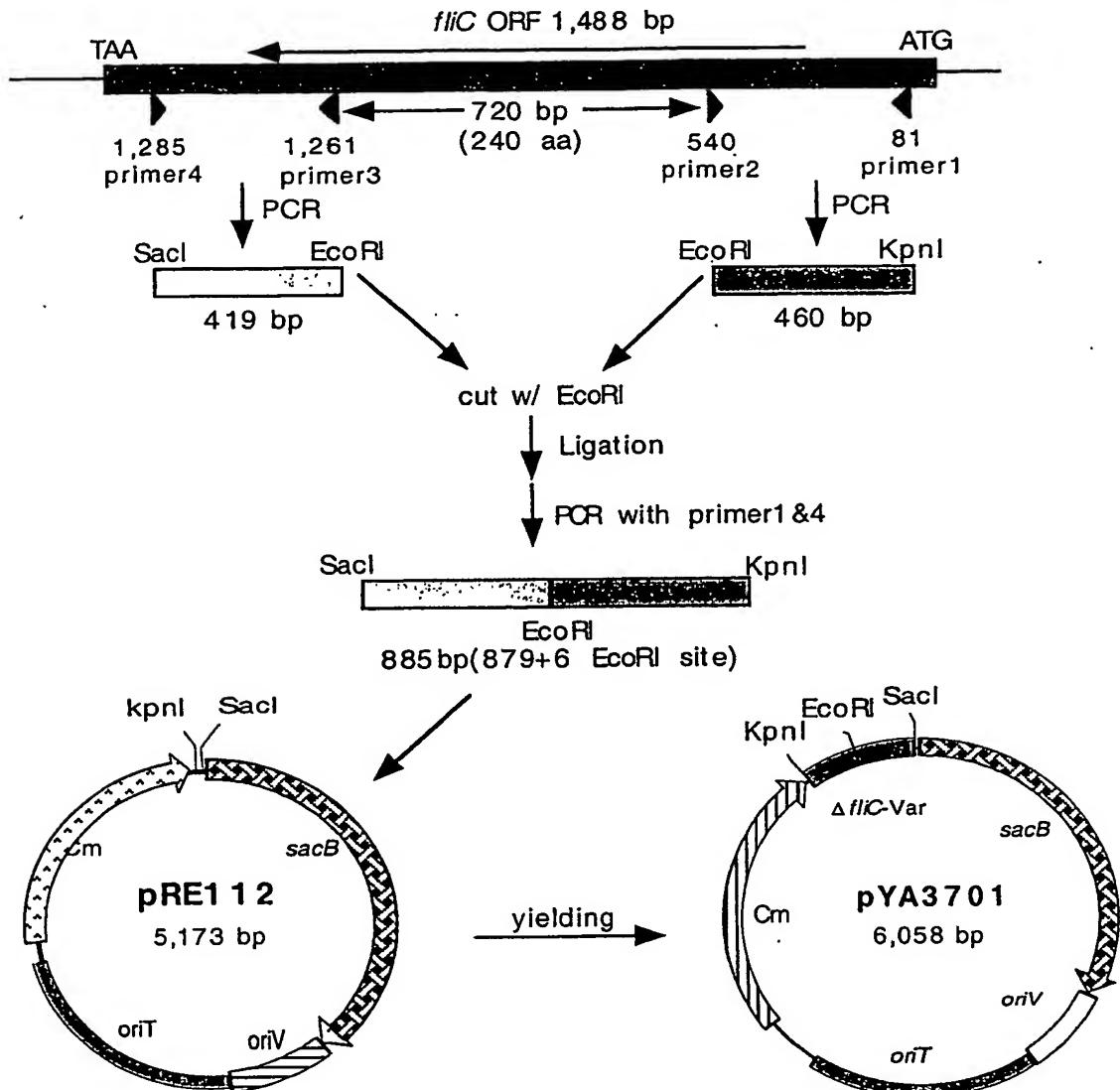
FIGURE 14. Construction of suicide vector for  $\Delta fliC825$ 

FIGURE 15. Construction of sulcid vector for  $\Delta f_{17}B217$

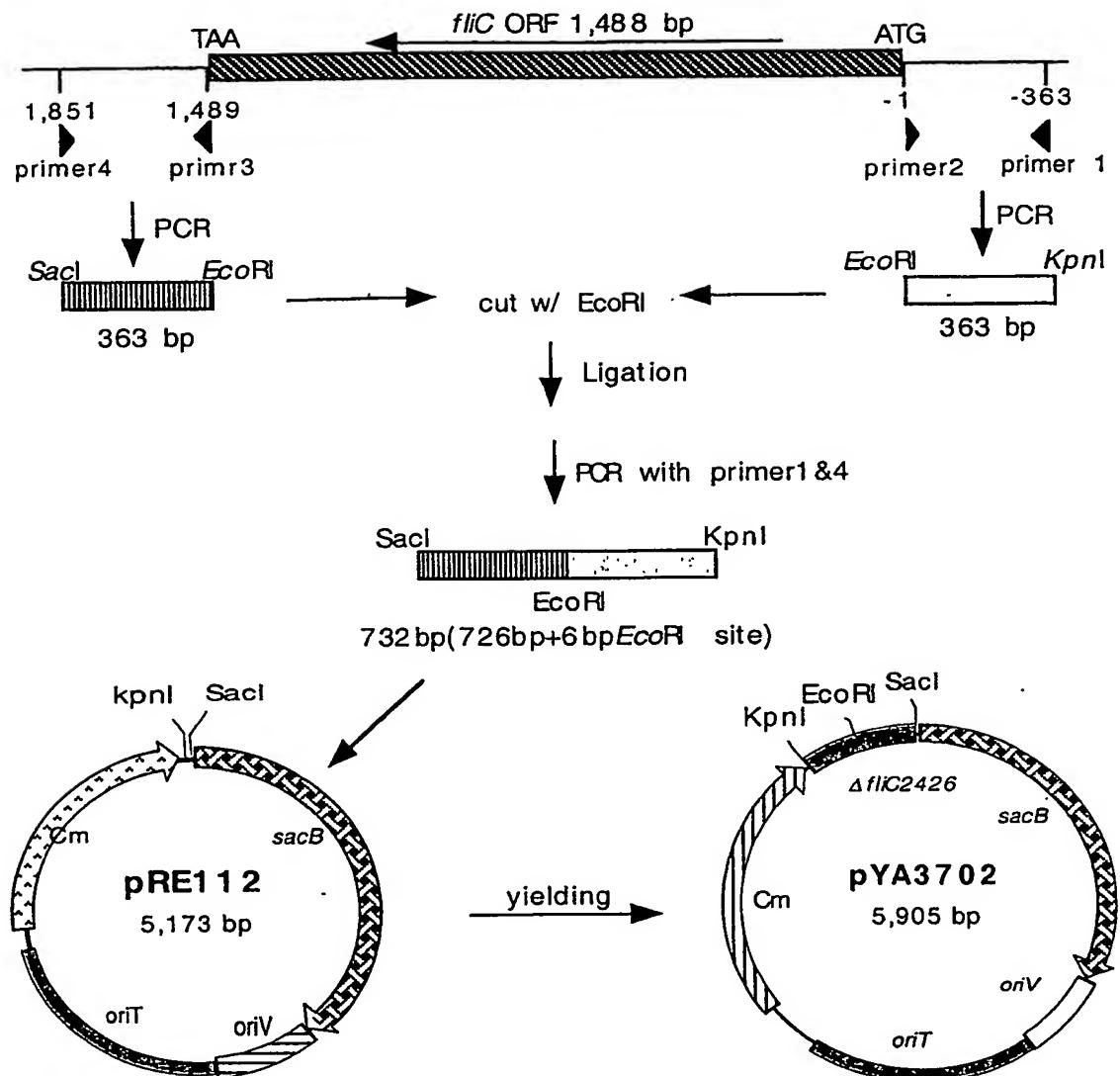


**FIGURE 16. *Salmonella typhimurium* SL1344 chromosomal deletions:****A.  $\Delta fliC825$** **B.  $\Delta fliB217$** 



primer 1: delV.fliC 1 *KpnI/b p81-104*  
 5'-GGGGTA CCCGCTATCGAGCGT CTG TCT TCCGG-3'  
 primer 2: delV fliC 2 *EcoRI/bp540-516*  
 5'-GGGAAT TCCTTA TAT TTTTGT TGCACATTCAAG-3'  
 primer 3: delV fliC 3 *EcoRI/b p1261-1285*  
 5'-GGGAAT TCACGTTACGTT CTGACCTGGGTGCG-3'  
 primer 4: delV fliC 4 *SacI/bp1679-1655*  
 5'-GGGAGCTCCGTCTTA TCCAGCGTGATT TTCCA-3'

Figure 17. Construction of a suicide vector for transfer of  $\Delta fliC$ -Var mutation

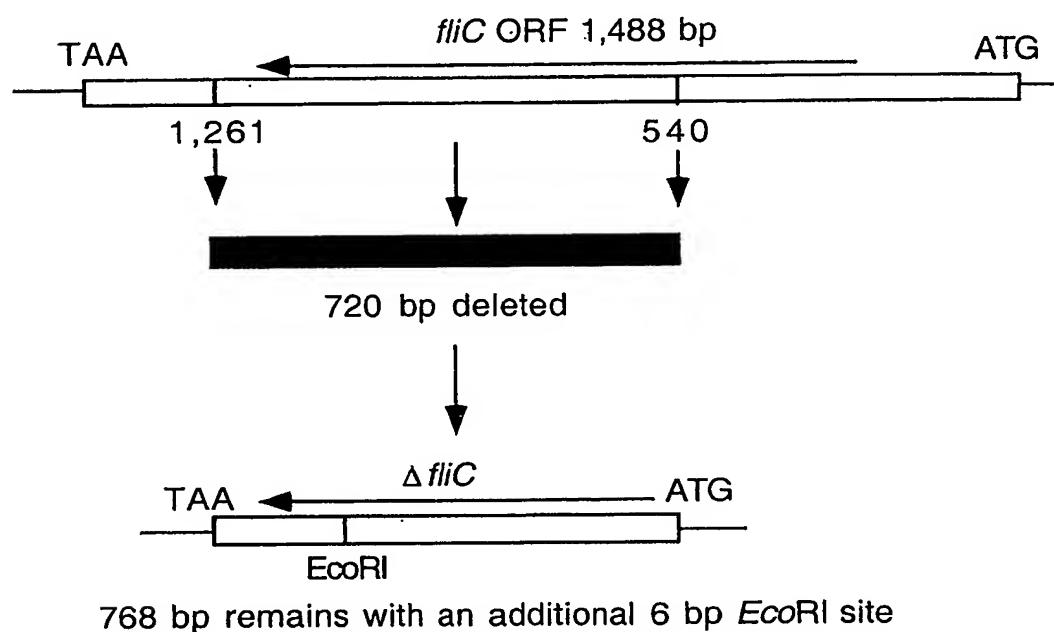


primer 5: *fliC* 1 *KpnI/b* p-363/- 339  
 5'-GGGGTA CCTGTT TGGTAA TTGGGG TTA AGCGT-3'  
 primer 6: *fliC* 2 *EcoRI/bp*-1/-24  
 5'-GGGAAT TCGATCTTTCCCTTA TCAATT ACAAC-3'  
 primer 7: *fliC* 3 *EcoRI/bp*1,489/ 1,513  
 5'-GGGAATTCTCGGGATTGATTCACCGACACCG-3'  
 primer 8: *fliC* 4 *SacI/b* p1,851/1 827  
 5'-GGGAGCTCGGAAAGAGAACAGGTATGACTT-3'

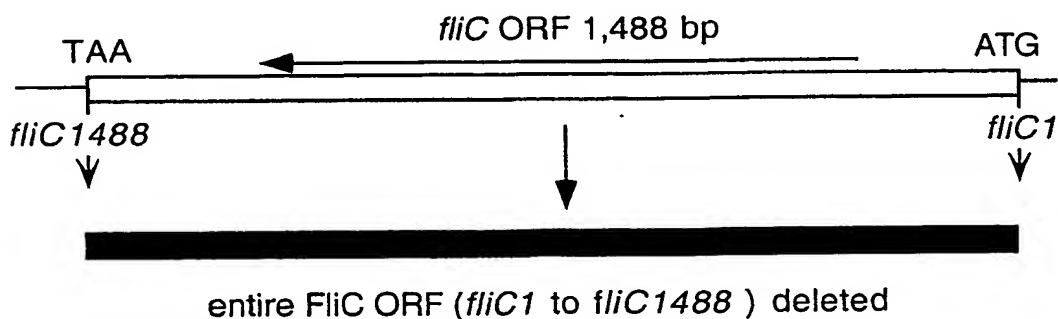
Figure 18. Construction of a suicide vector for transfer of  $\Delta fliC 2426$  mutation

Figure 19. *S. typhimurium* UK-1 chromosomal map for  $\Delta fliC$ -Var and  $\Delta fliC2426$  deletion mutations.

**A.  $\Delta fliC$ -Var**



**B.  $\Delta fliC2426$**



**Figure 20.** DNA nucleotide sequence of improved *araC\**  $P_{BAD}$  region in pYA3624.

**Figure 20. (cont'd)**

TCA CAT CTT TTC CGC CAG CAG TTA GGG ATT AGC GTC TTA AGC TGG CGC GAG GAC CAA CGC  
S H L F R Q Q L G I S V L S W R E D Q R  
  
ATT AGT CAG GCG AAG CTG CTT TTG AGC ACT ACC CGG ATG CCT ATC GCC ACC GTC GGT CGC  
I S Q A K L L S T T R M P I A T V G R  
  
AAT GTT GGT TTT GAC GAT CAA CTC TAT TTC TCG CGA GTA TTT AAA AAA TGC ACC GGG GCC  
N V G F D D Q L Y F S R V F K K C T G A  
  
AGC CCG AGC GAG TTT CGT GCC GGT TGT GAA GAA AAA GTG AAT GAT GTA GCC GTC AAG TTG  
S P S E F R A G C E E K V N D V A V K L  
  
TCA TAA TTG GTA ACG AAT CAG ACA ATT GAC GGC  
S \*  
←araC\* ends

Figure 21. DNA and amino acid sequences of *P<sub>fur</sub>* and *fur* gene of *S. paratyphi A*.

*fldA*

181/61 primer 211/71  
 GAA GCG CAA TGT GAC TGG GAT GAC TTC TTC CCG ACT CTC GAA GAG ATT GAC TTT AAC GGT  
 E A Q C D W D D F F P T L E E I D F N G

241/81 271/91  
 AAG CTG GTG GCG CTG TTT GGC TGT GGC GAT CAG GAA GAC TAC GCG GAA TAC TTC TGT GAT  
 K L V A L F G C G D Q E D Y A E Y F C D

301/101 331/111  
 GCG CTG GGC ACG ATT CGC GAC ATT ATT GAG CCG CGC GCC GCG ACG ATT CTG GGT CAC TGG  
 A L G T I R D I I E P R G A T I V G H W

361/121 391/131  
 CCA ACT GCA GGC TAT CAT TTT GAA GCC TCT AAA GGT CTG GCT GAC GAC GAT CAT TTT GTC  
 P T A G Y H F E A S K G L A D D D H F V

421/141 451/151  
 GGT CTG GCG ATT GAC GAA GAC CGT CAG CCT GAA CTG ACC GCC GAG CGT GTT GAA AAA TGG  
 G L A I D E D R Q P E L T A E R V E K W

481/161 511/171  
 GTT AAG CAA GTT TCG GCT GAA TTG CAC CTC GAC GAC ATC CTC AAC GCC TAA TCT TAT GCG  
 V K Q V S A E L H L D D I L N A \* ↑ *fldA* ends

541/181 571/191  
 GCG CAG CGT TAT ATC TGC GCC GCA TCA ATA GAC AAG ACC AAT CAA AAT TGC TAC AAA  
 primer ↘ delete (fur-253) OxyR binding site

601/201 631/211  
TTT GTA ACT TTC GCA CCC ATC CCT GTA CAA TGT CCG GGT GTA ATC AGG TGG CGC CAG A↑

661/221 691/231 -35  
TTG CAG GCA AAA CCA CAG TTT TAT TAA CAT CTG CGA GAG ACT TGC GGT TTT CAT TTC GGC  
 CRP binding site

721/241 -10 751/251  
 ATG GCA GTC CTA TAA TGA TAC GCA TTA TGT TGA GTG CAA TTT CTG TCA CTT CTC TAA TGA  
 Fur consensus

781/261 SD 813/1  
 AGT GAA TCG TTT AGC AAC AGG ACA GAT TCC GC ATG ACT GAC AAC AAT ACC GCA TTA AAG  
 delete (fur-15) ↘ primer M T D N N T A L K  
 fur starts ↑

840/10 873/21  
 AAG GCT GGC CTG AAA GTA ACG CTT CCT CGT TTA AAA ATT CTG GAA GTT CTT CAG GAA CCA  
 K A G L K V T L P R L K I L E V L Q E P

900/30 933/41  
 GAT AAC CAT CAC GTC AGT GCG GAA GAT TTA TAC AAA CGC CTG ATC GAC ATG GGT GAA GAA  
 D N H H V S A E D L Y K R L I D M G E E

960/50 993/61  
 ATC GGT CTG GCA ACC GTA TAC CGT GTG CTG AAC CAG TTT GAC GAT GCC GGT ATC GTG ACC  
 I G L A T V Y R V L N Q F D D A. G I V T

1020/70 1053/81  
 CGC CAT AAT TTT GAA GGC GGT AAA TCC GTT TTT GAA CTG ACG CAA CAG CAT CAT CAC GAC  
 R H N F E G G K S V F E L T Q Q H H H D

1080/90 primer 1113/101  
 CAT CTT ATC TGC CTT GAT TGC GGA AAA GTG ATT GAA TTT AGT GAT GAC TCT ATT GAA GCG  
 H L I C L D C G K V I E F S D D S I E A

1140/110 1173/121  
 CGC CAG CGT GAA ATT GCG GCG AAA CAC GGT ATT CGT TTA ACT AAT CAC AGC CTC TAT CTT  
 R Q R E I A A K H G I R L T N H S L Y L

1200/130 1233/141  
 TAC GGC CAC TGC GCT GAA GGC GAC TGC CGC GAA GAC GAG CAC GCG CAC GAT GAC GCG ACT  
 Y G H C A E G D C R E D E H A H D D A T

1260/150  
 AAA TAA  
 K \* fur ends

**Figure 22. Construction of the suicide vector to introduce new  $\Delta P_{fur-33}::TT$  araC  $P_{BAD}$  fur deletion-insertion mutation.**

Oligo 9 (T4 ipIII TT-N) : 5' CCTGGTACCTAGGCCTCTAGATAAATAAAAGCAGTTACAACCTCTAGAATTGTG  
AATATATTATCACAAATTCTAGGATAGAATAATAAAAGATCTCTGCAGGGC 3'

Oligo 10 (T4 ipIII TT-C) : 5' GCCCTGCAGAGATCTTTATTATTCTATCTAGAATTGTGATAATATATTCACAA  
TTCTAGGAGTTGTAAGACTGCTTTATTCTAGAGGCCTAGGTACCCAGG 3'

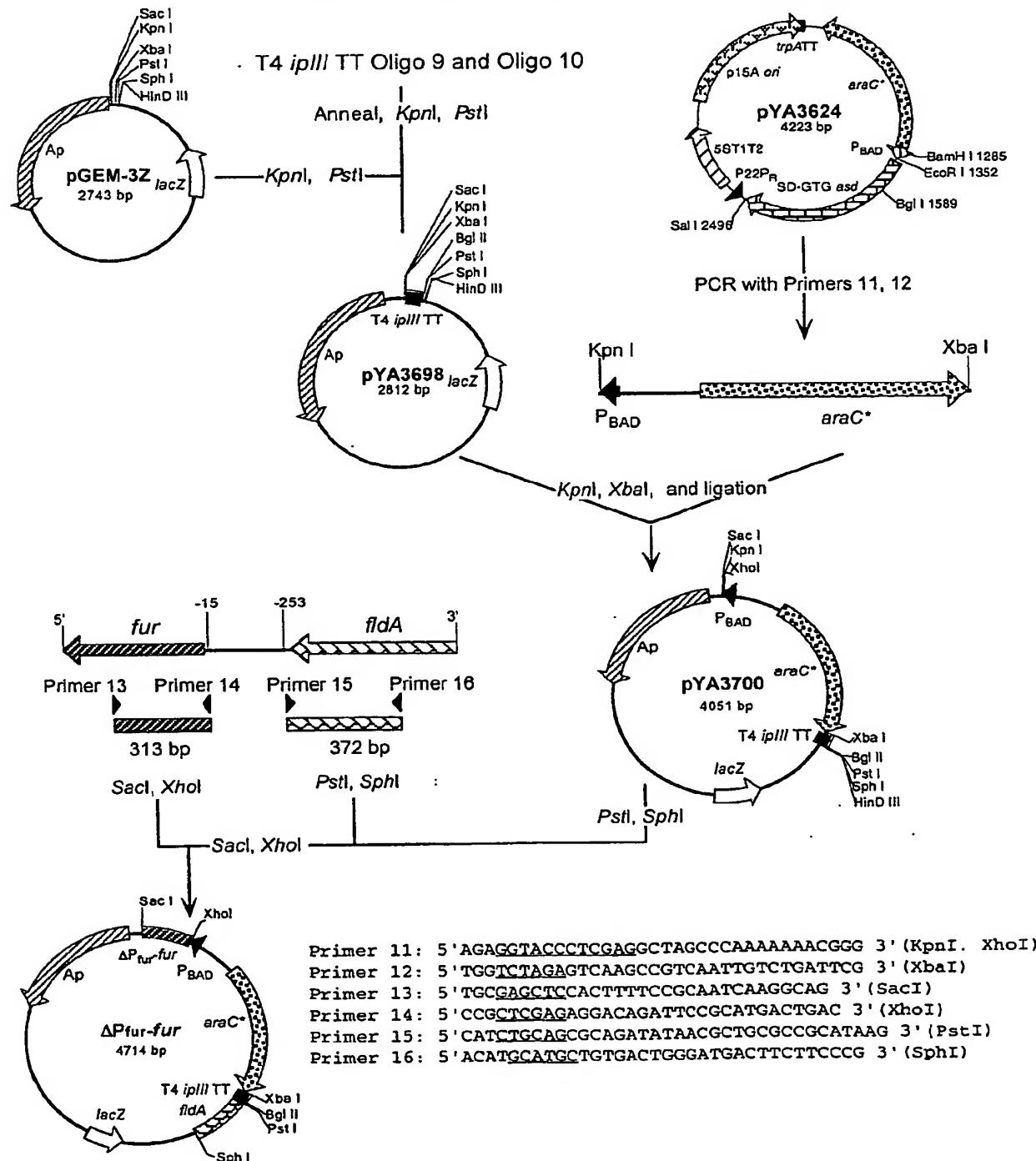


Figure 22. (cont'd)

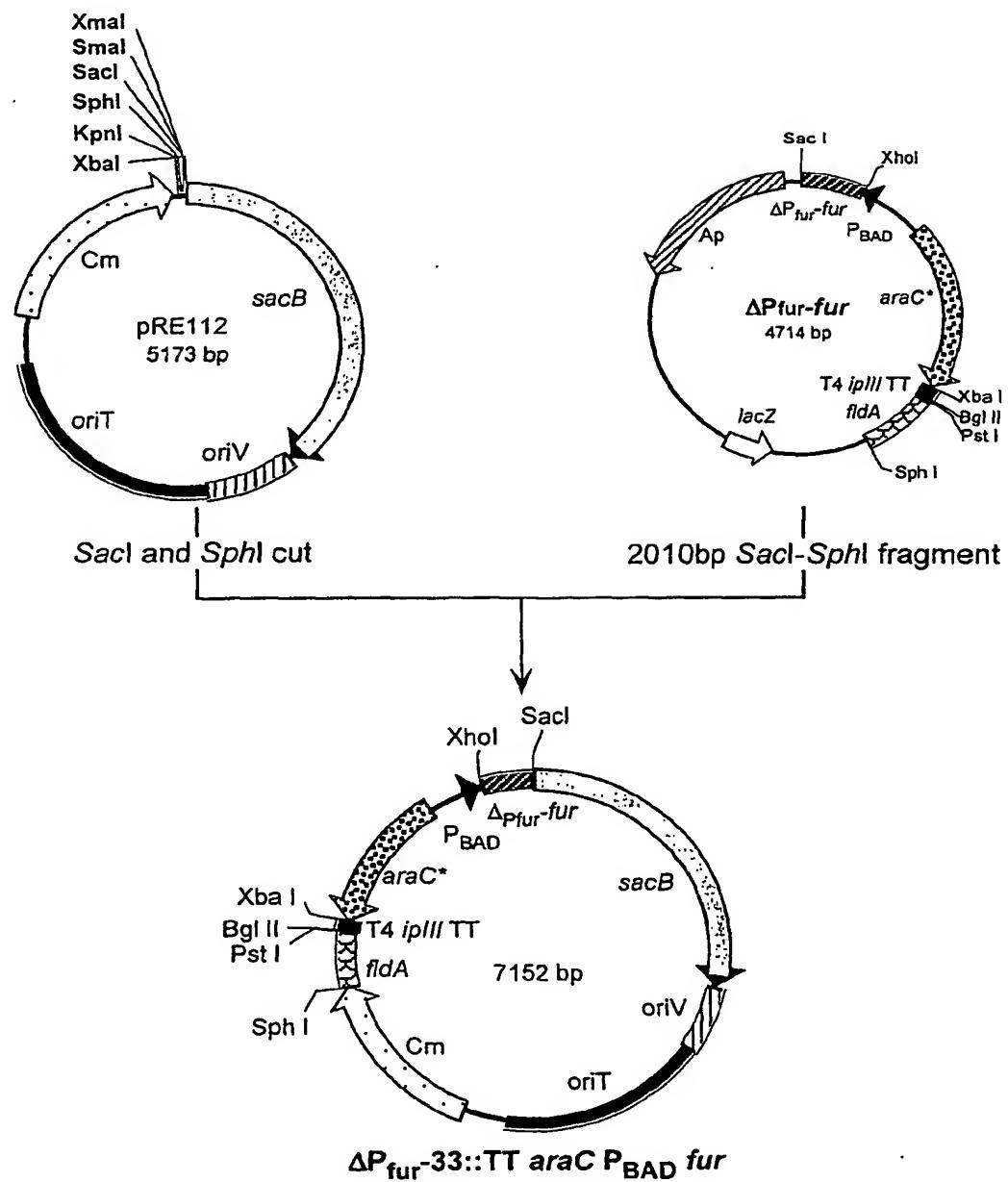


Figure 23. Chromosomal map of  $\Delta P_{fur}$ -33::TT *araC*  $P_{BAD}$  *fur* deletion-insertion mutation.

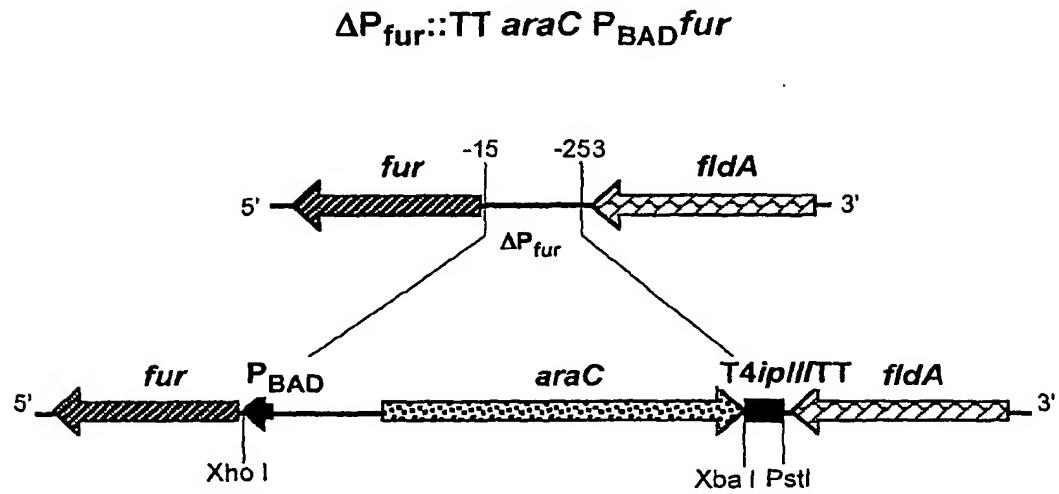


Figure 24. DNA sequence of the  $\Delta P_{fur}$ -33::TT araC\*  $P_{BAD fur}$ .

Figure 24. (cont'd.)

Figure 24. (cont'd)

N F E G G K S V F E L T Q Q H H H D H L  
AAT TTT GAA GGC GGT AAA TCC GTT TTT GAA CTG ACG CAA CAG CAT CAT CAC GAC CAT CTT  
TTA AAA CTT CCG CCA TTT AGG CAA AAA CTT GAC TGC GTT GTC GTA GTA GTG CTG GTA GAA

I C L D C G K V I E F S D D S I E A R Q  
ATC TGC CTT GAT TGC GGA AAA GTG ATT GAA TTT AGT GAT GAC TCT ATT GAA GCG CGC CAG  
TAG ACG GAA CTA ACG CCT TTT CAC TAA CTT AAA TCA CTA CTG AGA TAA CTT CGC GCG GTC

R E I A A K H G I R L T N H S L Y L Y G  
CGT GAA ATT GCG GCG AAA CAC GGT ATT CGT TTA ACT AAT CAC AGC CTC TAT CTT TAC GGC  
GCA CTT TAA CGC CGC TTT GTG CCA TAA GCA AAT TGA TTA GTG TCG GAG ATA GAA ATG CCG

H C A E G D C R E D E H A H D D A T K \* ←fur ends  
CAC TGC GCT GAA GGC GAC TGC CGC GAA GAC GAG CAC GCG CAC GAT GAC GCG ACT AAA TAA  
GTG ACG CGA CTT CCG CTG ACG GCG CTT CTG CTC GTG CGC GTG CTA CTG CGC TGA TTT ATT

**Figure 25.** DNA and amino acid sequences of *P<sub>rpos</sub>*, *rpos* and flanking region of *S. typhimurium* and *S. typhi*.

STM: *S. typhimurium* 14028S  
STY: *S. typhi* CT18

AAT	GCA	AGC	AGT	ACG	TCA	ACC	AGC	GCG	CCG	ATT	TCC	GCA	TGG	CGC	TGG	CCG	ACG	GAT	GGC-STM
AAT	GCA	AGC	AGT	ACG	TCA	ACC	AGC	GCG	CCG	ATT	TCC	GCA	TGG	CGC	TGG	CCG	ACG	GAT	GGC-STY
N	A	S	S	T	S	T	S	A	P	I	S	A	W	R	W	P	T	D	G

AAA GTG ATC GAA AAC TTT GGC GCT TCC GAA GGG GGC AAT AAA GGG ATC GAC ATT GCA GGC  
AAA GTG ATC GAA AAC TTT GGC GCT TCC GAA GGG GGC AAT AAA GGG ATC GAC ATT GCA GGC  
K V I E N F G A S E G G N K G I D I A G

AGT	AAG	GGA	CAG	GCT	ATC	GTC	GCA	ACC	GCT	GAT	GGG	CGC	GTC	GTA	TAT	GCC	GGT	AAC	GCA
AGT	AAG	GGA	CAG	GCT	ATC	GTC	GCA	ACC	GCT	GAT	GGG	CGC	GTC	GTA	TAT	GCC	GGT	AAC	GCA
S	K	G	O	A	I	V	A	T	A	D	G	R	V	V	Y	A	G	N	A

CTG CGT GGT TAC GGT AAT CTT ATT ATC ATC AAA CAT AAC GAT GAT TAC CTG AGT GCC TAC  
 CTG CGT GGT TAC GGT AAT CTT ATT ATC ATC AAA CAT AAC GAT GAT TAC CTG AGT GCC TAC  
 L R G X G N L I I I K H N D D Y L S A Y

GCC	CAT	AAT	GAT	ACG	ATG	CTG	GTC	CGG	GAA	CAA	CAG	GAA	GTT	AAG	GCG	GGG	CAA	AAA	ATC
GCC	CAT	AAT	GAT	ACG	ATG	CTG	GTC	CGG	GAA	CAA	CAG	GAA	GTT	AAG	GCG	GGG	CAA	AAA	ATC
A	H	N	D	T	M	L	V	R	E	O	O	E	V	K	A	G	O	K	I

GCT	ACT	ATG	GGT	AGC	ACC	GGC	ACC	AGC	TCT	ACA	CGC	TTG	CAT	TTT	GAA	ATT	CGT	TAC	AAG
GCT	ACT	ATG	GGT	AGC	ACC	GGC	ACC	AGC	TCT	ACA	CGC	TTG	CAT	TTT	GAA	ATT	CGT	TAC	AAG
A	T	M	G	S	T	G	T	S	S	T	R	L	H	F	E	I	R	Y	K

```

GGG AAA TCC GTA AAC CCG CTG CGT TAT TTA CCG CAG CGA TAA AG
GGG AAA TCC GTA AAC CCG CTG CGT TAT TTA CCG CAG CGA TAA AG
G K S V N P L R Y L P Q R *

```

← *nlpD* ends

SD

```

CGG CGG AAC CAG GCT TTG ACT TGC TAG TTC CGT CAA GGG ATC ACG GGT AGG AGC CAC CTT
CGG CGG AAC CAG GCT TTG ACT TGC TAG TTC CGT CAA GGG ATC ACG GGT AGG AGC CAC CTT

```

*rpos*<sub>-48</sub>       $\Delta P_{rpos}$  (*rpos*-48 to -13 deleted)      *rpos*<sub>-13</sub>

```

1185/1          1215/11
ATG AGT CAG AAT ACG CTG AAA GTT CAT GAT TTA AAT GAA GAC GCG GAA TTT GAT GAG AAC-STM
ATG AGT CAG AAT ACG CTG AAA GTT CAT GAT TTA AAT GAA GAC GCG GAA TTT GAT GAG AAC-STY
M   S   Q   N   T   L   K   V   H   D   L   N   E   D   A   E   F   D   E   N
rpoS starts →

```

1245/21 1275/31  
GGA GTA GAG GCT TTT GAC GAA AAA GCC TTG AGT GAA GAG GAA CCC AGT GAT AAC GAC CTG  
GGA GTA GAG GCT TTT GAC GAA AAA GCC TTG AGT GAA GAG GAA CCC AGT GAT AAC GAC CTG  
G V E A F D E K A L S E E E P S D N D L

1305/41	1335/51
GCT GAA GAA GAG CTG TTA TCG CAA GGG GCC	ACA CAG CGT GTG TTG GAC GCG ACT CAG CTT
GCT GAA GAA GAG CTG TTA TCG CAA GGG GCC	ACA CAG CGT GTG TTG GAC GCG ACT CAG CTT
A E E E L L S Q G A T Q R V L D A T Q L	

1365/61	1395/71
TAC CTT GGT GAG ATT GGG TAT TCA CCA CTG TTA ACA GCC GAA GAA GAA GTC TAT TTT GCG	TAC CTT GGT GAG ATT GGG TAT TCA CCA CTG TTA ACA GCC GAA GAA GAA GTC TAT TTT GCG
Y L G E I G Y S P L L T A E E E E V Y F A	

1425/81 1455/91  
CGT CGC GAC CTG CGT GGA GAT GTC GCT TCT CGC CGT CGC ATG ATT GAG AGT AAC CTG CGT  
CGT CGC GCA CTG CGT GGA GAT GTC GCT TCT CGC CGT CGC ATG ATT GAG AGT AAC CTG CGT

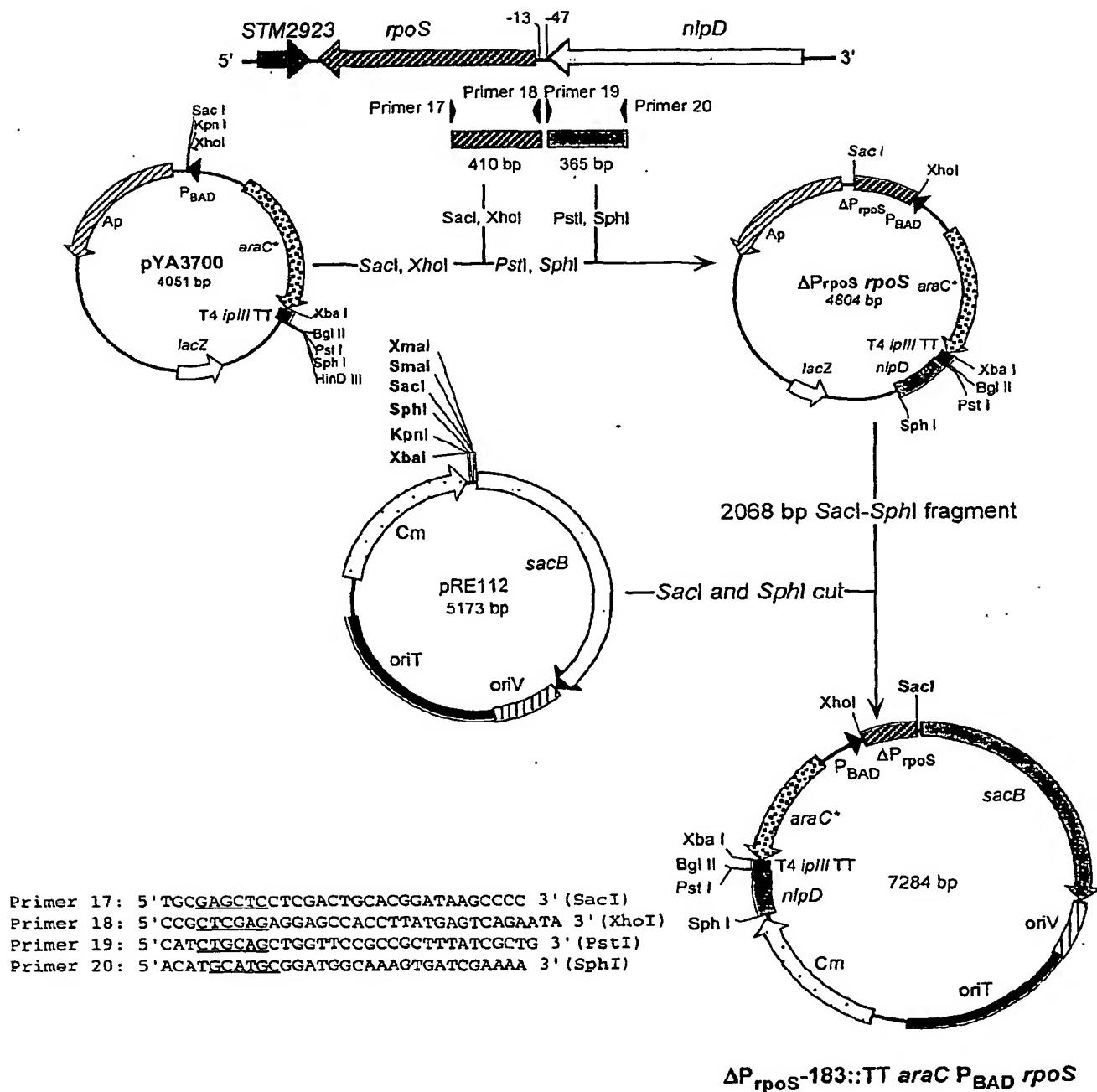
R	R	A	L	R	G	D	V	A	S	R	R	R	M	I	E	S	N	L	R
1485/101										1515/111									
CTG	GTG	GTA	AAA	ATT	GCC	CGC	CGT	TAT	GGC	AAT	CGT	GGA	CTG	GCG	TTG	CTG	GAC	CTG	ATT
CTG	GTG	GTA	AAA	ATT	GCC	CGC	CGT	TAT	GGC	AAT	CGT	GGA	CTG	GCG	TTG	CTG	GAC	CTG	ATT

Figure 25. (cont'd)

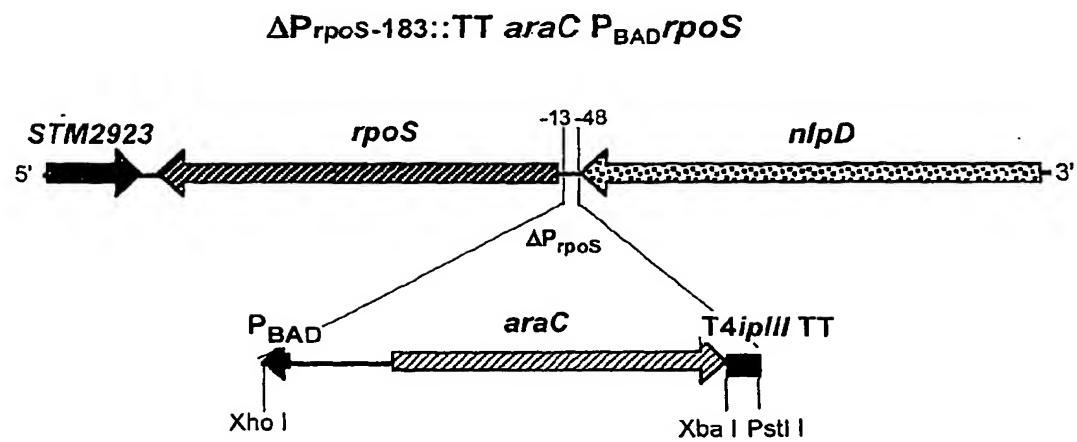
1545/121 1575/131  
 GAA GAG GGC AAC CTG GGG CTT ATC CGT GCA GTC GAG AAG TTT GAC CCG GAA CGC GGG TTC  
 GAA GAG GGC AAC CTG GGG CTT ATC CGT GCA GTC GAG AAG TTT GAC CCG GAA CGC GGG TTC  
 E E G N L G L I R A V E K F D P E R G F  
 1605/141 1635/151  
 CGC TTC TCA ACA TAC GCA ACC TGG TGG ATT CGC CAG ACA ATC GAA CGG GCG ATC ATG AAC  
 CGC TTC TCA ACA TAC GCA ACC TGG TGG ATT CGC CAG ACA ATC GAA CGG GCG ATT ATG AAC  
 R F S T Y A T W W I R Q T I E R A I M N  
 1665/161 1695/171  
 CAA ACC CGT ACG ATT CGC TTG CCG ATT CAC ATT GTT AAA GAG CTG AAC GTA TAC CTG CGC  
 CAA ACC CGT ACG ATT CGC TTG CCG ATT CAC ATT GTT AAA GAG CTG AAC GTA TAC CTG CGC  
 Q T R T I R L P I H I V K E L N V Y L R  
 1725/181 1755/191  
 ACC GCA CGT GAG TTG TCG CAT AAA CTG GAC CAC GAA CCG AGT GCG GAA GAA ATT GCA GAG  
 ACC GCA CGT GAG TTG TCG CAT AAA CTG GAC CAC GAA CCG AGT GCG GAA GAA ATT GCA GAG  
 T A R E L S H K L D H E P S A E E I A E  
 1785/201 1815/211  
 CAA CTG GAT AAA CCG GTT GAT GAC GTC AGC CGT ATG CTT CGT CTC AAC GAG CGC ATT ACC  
 CAA CTG GAT AAA CCG GTT GAT GAC GTC AGC CGT ATG CTT CGT CTC AAC GAG CGC ATT ACC  
 Q L D K P V D D V S R M L R L N E R I T  
 1845/221 1875/231  
 TCG GTA GAC ACC CCG CTG GGC GGT GAT TCC GAA AAA GCG TTG CTG GAC ATC CTG GCC GAT  
 TCG GTA GAC ACC CCG CTG GGC GGT GAT TCC GAA AAA GCG TTG CTG GAC ATC CTG GCC GAT  
 S V D T P L G G D S E K A L L D I L A D  
 1905/241 1935/251  
 GAA AAA GAG AAC GGT CCG GAA GAC ACC ACG CAA GAT GAC GAT ATG AAA CAG AGC ATC GTC  
 GAA AAA GAG AAC GGT CCG GAA GAC ACC ACG CAA GAT GAC GAT ATG AAA CAG AGC ATC GTC  
 E K E N G P E D T T Q D D D M K Q S I V  
 1965/261 1995/271  
 AAA TGG TTG TTC GAA CTG AAC GCC AAA CAG CGT GAA GTG CTG GCG CGC CGT TTC GGT CTG  
 AAA TGG TTG TTC GAA CTG AAC GCC AAA CAG CGT GAA GTG CTG GCG CGC CGT TTC GGT CTG  
 K W L F E L N A K Q R E V L A R R F G L  
 2025/281 2055/291  
 CTG GGA TAT GAA GCT GCG ACA CTG GAA GAT GTC GGC CGT GAA ATC GGT CTT ACG CGT GAA  
 CTG GGA TAT GAA GCT GCG ACA CTG GAA GAT GTC GGC CGT GAA ATC GGT CTT ACG CGT GAA  
 L G Y E A A T L E D V G R E I G L T R E  
 2085/301 2115/311  
 CGT GTT CGT CAG ATT CAG GTT GAA GGC CTG CGC CGT CTG CGC GAA ATT CTG CAG ACG CAG  
 CGT GTT CGT CAG ATT CAG GTT GAA GGC CTG CGC CGT CTG CGC GAA ATT CTG CAG ACG CAG  
 R V R Q I Q V E G L R R L R E I L Q T Q  
 2145/321 2175/331  
 GGG CTG AAT ATC GAA GCG CTG TTC CGC GAG TAA GTA CCC TTG TCA  
 GGG CTG AAT ATC GAA GCG CTG TTC CGC GAG TAA GTA CCC TTG TCA  
 G L N I E A L F R E \*

← rpoS ends

**Figure 26. Construction of suicide vector for introducing  $\Delta P_{rpoS-183::TT}$  *araC*  $P_{BAD}$  *rpoS* deletion-insertion mutation.**



**Figure 27. Chromosomal map of  $\Delta P_{rpoS}$ -183::TT *araC*  $P_{BAD}$  *rpoS* deletion-insertion mutation.**



*rpoS* promoter region (-13 to -48) deleted and 1344 bp  $P_{BAD}$  *araC* TT inserted.

**Figure 28. DNA and amino acid sequences of the *S. typhimurium*  $P_{phoPQ}$  and  $phoPQ$  and the flanking region.**

**PurB/as1 →**

ATT GCG TTG AAC CAT TTC AAA CAG AAA ACC ATC GCC GGG GAG ATC GGT TCT TCT ACC ATG  
 I A L N H F K Q K T I A G E I G S S T M  
 CCG CAT AAA GTT AAC CCC ATT GAC TTT GAA AAC TCA GAA GGC AAC CTC GGT CTG TCT AAC  
 P H K V N P I D F E N S E G N L G L S N  
 GCA GTG TTG CAC CAT CTG GCA AAC AAA CTG CCG GTT TCC CGC TGG CAG CGC GAT CTG ACC  
 A V L H H L A N K L P V S R W Q R D L T  
 GAC TCA ACC GTC CTG CGT AAC CTG GGT GTC GGC ATC GGC TAT GCG CTT ATC GCT TAT CAG  
 D S T V L R N L G V G I G Y A L I A Y Q  
 TCC ACC CTG AAG GGC GTC AGC AAG CTG GAA GTC AAC CGC GAT CAT CTG CTT GAC GAA CTG  
 S T L K G V S K L E V N R D H L L D E L  
 GAT CAC AAC TGG GAA GTC TTA GCC GAA CCG ATC CAG ACC GTC ATG CGC CGC TAT GGT ATT  
 D H N W E V L A E P I Q T V M R R Y G I  
 GAA AAA CCA TAT GAA AAA CTG AAA GAG TTG ACC CGT GGC AAG CGT GTT GAT GCC GAA GGA  
 E K P Y E K L K E L T R G K R V D A E G  
 ATG AAA CAG TTT ATT GAT AGT CTG GCC CTG CCG GAA GCA GAA AAA ACG CGC CTT AAA GCC  
 M K Q F I D S L A L P E A E K T R L K A  
 ATG ACG CCG GCA AAT TAT ATC GGT CGC GCT GTG ACT CTG GTC GAC GAA CTT AAA TAA TGC  
 M T P A N Y I G R A V T L V D E L K \*  
 ←purB ends

CTG CCT CAC CCT CTT TTC TTC AGA AAG AGG GTG ACT ATT TGT CTG GTT TAT TAA CTG TTT  
 ↑  
 phoPQ-109

Δ $P_{phoPQ}$  (phoPQ-109 to phoPQ-12 deleted)

ATC CCC AAA GCA CCA TAA TCA ACG CTA GAC TGT TCT TAT TGT TAA CAC AAG GGA GAA GAG  
 ↑ SD  
 phoPQ-12

723/1 753/11  
ATG ATG CGC GTC CTG GTT GTC GAG GAT AAT GCA TTA TTA CGC CAC CAC CTG AAG GTT CAG  
 M M R V L V V E D N A L L R H H L K V Q  
 phoP starts →

783/21 813/31  
 CTC CAG GAT TCA GGT CAC CAG GTC GAT GCC GCA GAA GAT GCC AGG GAA GCT GAT TAC TAC  
 L Q D S G H Q V D A A E D A R E A D Y Y  
 843/41 873/51  
 CTT AAT GAA CAC CTT CCG GAT ATC GCT ATT GTC GAT TTA GGT CTG CCG GAT GAA GAC GGC  
 L N E H L P D I A I V D L G L P D E D G  
 903/61 933/71  
 CTT TCC TTA ATA CGC CGC TGG CGC AGC AGT GAT GTT TCA CTG CCG GTT CTG GTG TTA ACC  
 L S L I R R W R S S D V S L P V L V L T  
 963/81 993/91  
 GCG CGC GAA GGC TGG CAG GAT AAA GTC GAG GTT CTC AGC TCC GGG GCC GAT GAC TAC GTG  
 A R E G W Q D K V E V L S S G A D D Y V  
 1023/101 1053/111  
 ACG AAG CCA TTC CAC ATC GAA GAG GTA ATG GCG CGT ATG CAG GCG TTA ATG CGC CGT AAT  
 T K P F H I E E V M A R M Q A L M R R N

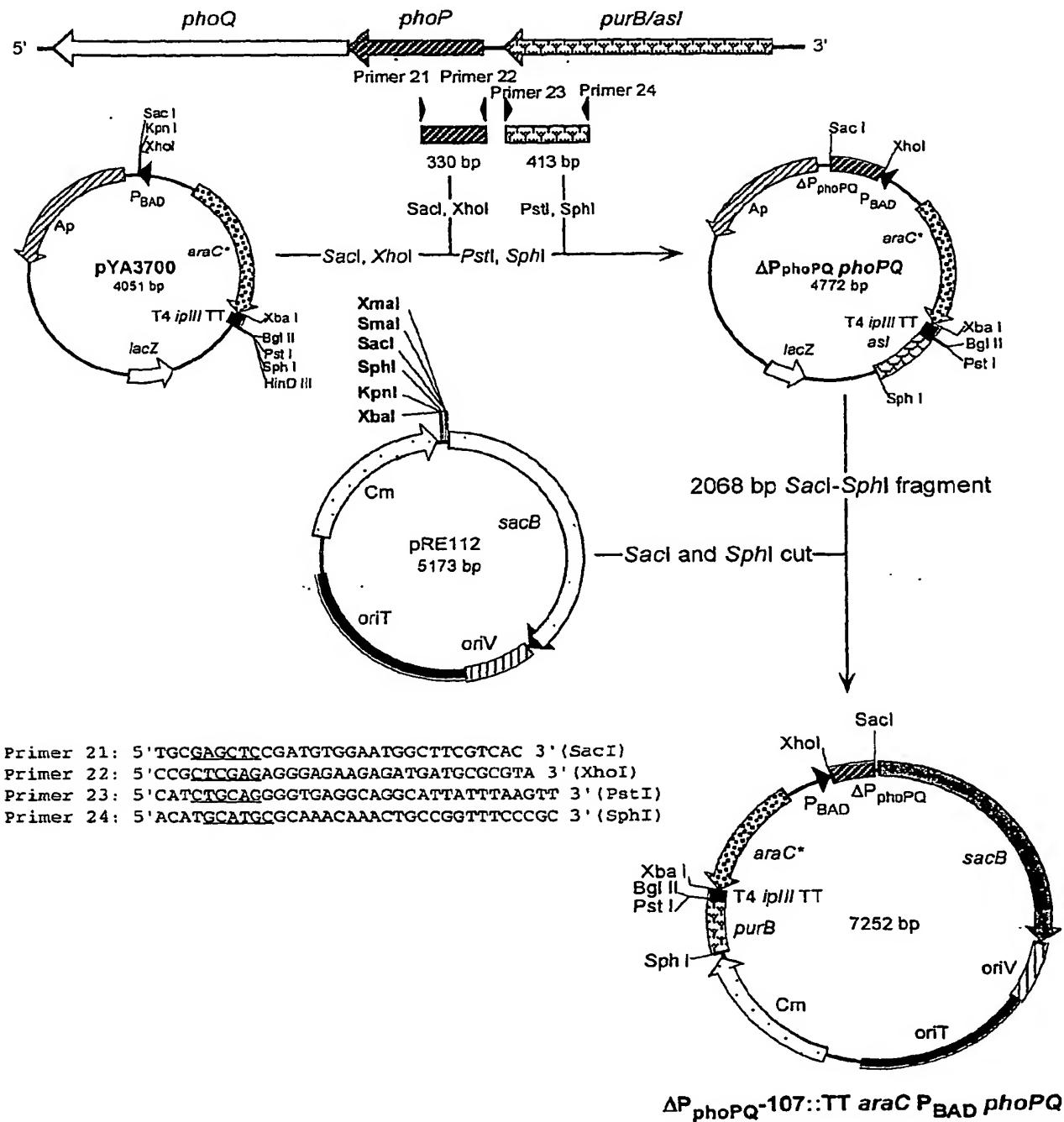
Figure 28. (cont'd)

1083/121 1113/131  
 AGC GGT CTG GCC TCC CAG GTG ATC AAC ATC CCG CCG TTC CAG GTG GAT CTC TCA CGC CGG  
 S G L A S Q V I N I P P F Q V D L S R R  
 1143/141 1173/151  
 GAA TTA TCC GTC AAT GAA GAG GTC ATC AAA CTC ACG GCG TTC GAA TAC ACC ATT ATG GAA  
 E L S V N E E V I K L T A F E Y T I M E  
 1203/161 1233/171  
 ACG CTT ATC CGT AAC AAC GGT AAA GTG GTC AGC AAA GAT TCG CTG ATG CTT CAG CTG TAT  
 T L I R N N G K V V S K D S L M L Q L Y  
 1263/181 1293/191  
 CCG GAT GCG GAA CTG CGG GAA AGT CAT ACC ATT GAT GTT CTC ATG GGG CGT CTG CGG AAA  
 P D A E L R E S H T I D V L M G R L R K  
 1323/201 1353/211  
 AAA ATA CAG GCC CAG TAT CCG CAC GAT GTC ATT ACC ACC GTA CGC GGA CAA GGA TAT CTT  
 K I Q A Q Y P H D V I T T V R G Q G Y L  
 1383/221 ← *phoP* ends  
 TTT GAA TTG CGC TAA TGA  
 F E L R \* \* \*  
*phoQ* starts → 1415/11  
 ATG AAT AAA TTT GCT CGC CAT TTT CTG CCG CTG TCG CTG CGG GTT CGT  
 M N K F A R H F L P L S L R V R  
 1445/21 1475/31  
 TTT TTG CTG GCG ACA GCC GGC GTC GTG CTG GTG CTT TCT TTG GCA TAT GGC ATA GTG GCG  
 F L L A T A G V V L V L S L A Y G I V A  
 1505/41 1535/51  
 CTG GTC GGC TAT AGC GTA AGT TTT GAT AAA ACC ACC TTT CGT TTG CTG CGC GGC GAA AGC  
 L V G Y S V S F D K T T F R L L R G E S  
 1565/61 1595/71  
 AAC CTG TTT TAT ACC CTC GCC AAA TGG GAA AAT AAT AAA ATC AGC GTT GAG CTG CCT GAA  
 N L F Y T L A K W E N N K I S V E L P E  
 1625/81 1655/91  
 AAT CTG GAC ATG CAA AGC CCG ACC ATG ACG CTG ATT TAC GAT GAA ACG GGC AAA TTA TTA  
 N L D M Q S P T M T L I Y D E T G K L L  
 1685/101 1715/111  
 TGG ACG CAG CGC AAC ATT CCC TGG CTG ATT AAA AGC ATT CAA CCG GAA TGG TTA AAA ACG  
 W T Q R N I P W L I K S I Q P E W L K T  
 1745/121 1775/131  
 AAC GGC TTC CAT GAA ATT GAA ACC AAC GTA GAC GCC ACC AGC ACG CTG TTG AGC GAA GAC  
 N G F H E I E T N V D A T S T L L S E D  
 1805/141 1835/151  
 CAT TCC GCG CAG GAA AAA CTC AAA GAA GTA CGT GAA GAT GAC GAT GAT GCC GAG ATG ACC  
 H S A Q E K L K E V R E D D D A E M T  
 1865/161 1895/171  
 CAC TCG GTA GCG GTA AAT ATT TAT CCT GCC ACG GCG CGG ATG CCG CAG TTA ACC ATC GTG  
 H S V A V N I Y P A T A R M P Q L T I V  
 1925/181 1955/191  
 GTG GTC GAT ACC ATT CCG ATA GAA CTA AAA CGC TCC TAT ATG GTG TGG AGC TGG TTC GTA  
 V V D T I P I E L K R S Y M V W S W F V  
 1985/201 2015/211  
 TAC GTG CTG GCC GCC AAT TTA CTG TTA GTC ATT CCT TTA CTG TGG ATC GCC GCC TGG TGG  
 Y V L A A N L L L V I P L L W I A A W W  
 2045/221 2075/231  
 AGC TTA CGC CCT ATC GAG GCG CTG GCG CGG GAA GTC CGC GAG CTT GAA GAT CAT CAC CGC  
 S L R P I E A L A R E V R E L E D H H R  
 2105/241 2135/251  
 GAA ATG CTC AAT CCG GAG ACG ACG CGT GAG CTG ACC AGC CTT GTG CGC AAC CTT AAT CAA  
 E M L N P E T T R E L T S L V R N L N Q  
 2165/261 2195/271

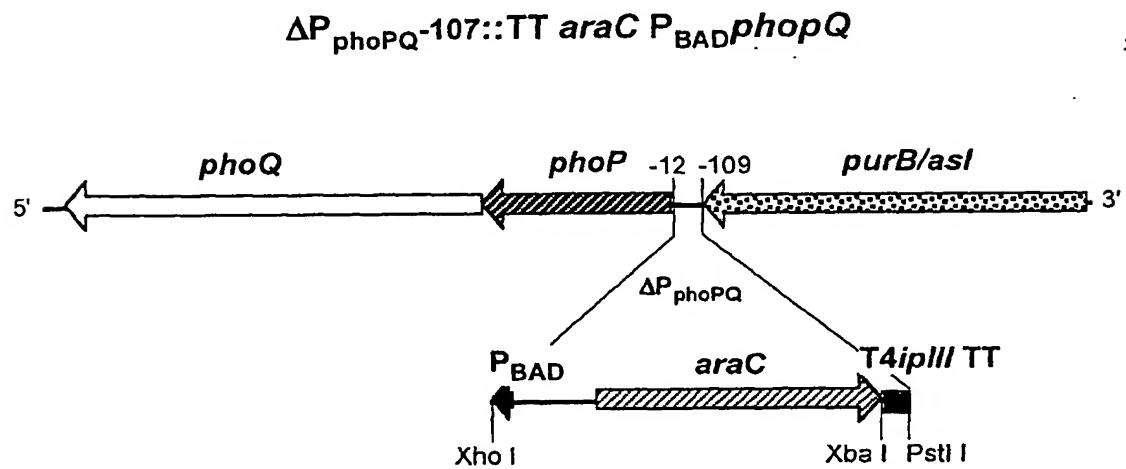
Figure 28. (cont'd)

CTG CTC AAA AGC GAG CGT GAA CGT TAT AAC AAA TAC CGC ACG ACC CTG ACC GAC CTG ACG  
 L L K S E R E R Y N K Y R T T L T D L T  
 2225/281 2255/291  
 CAC AGT TTA AAA ACG CCG CTC GCG GTT TTG CAG AGT ACG TTA CGC TCT TTA CGC AAC GAA  
 H S L K T P L A V L Q S T L R S L R N E  
 2285/301 2315/311  
 AAG ATG AGC GTC AGC AAA GCT GAA CCG GTG ATG CTG GAA CAG ATC AGC CGG ATT TCC CAG  
 K M S V S K A E P V M L E Q I S R I S Q  
 2345/321 2375/331  
 CAG ATC GGC TAT TAT CTG CAT CGC GCC AGT ATG CGC GGT AGC GGC GTG TTG TTA AGC CGC  
 Q I G Y Y L H R A S M R G S G V L L S R  
 2405/341 2435/351  
 GAA CTG CAT CCC GTC GCG CCG TTG TTA GAT AAC CTG ATT TCT GCG CTA AAT AAA GTT TAT  
 E L H P V A P L L D N L I S A L N K V Y  
 2465/361 2495/371  
 CAG CGT AAA GGG GTG AAT ATC AGT ATG GAT ATT TCA CCA GAA ATC AGT TTT GTC GGC GAG  
 Q R K G V N I S M D I S P E I S F V G E  
 2525/381 2555/391  
 CAA AAC GAC TTT GTC GAA GTG ATG GGC AAC GTA CTG GAC AAC GCT TGT AAA TAT TGT CTG  
 Q N D F V E V M G N V L D N A C K Y C L  
 2585/401 2615/411  
 GAG TTT GTC GAG ATT TCG GCT CGC CAG ACC GAC GAT CAT TTG CAT ATT TTC GTC GAA GAT  
 E F V E I S A R Q T D D H L H I F V E D  
 2645/421 2675/431  
 GAC GGC CCA GGC ATT CCC CAC AGC AAA CGT TCC CTG GTG TTT GAT CGC GGT CAG CGC GCC  
 D G P G I P H S K R S L V F D R G Q R A  
 2705/441 2735/451  
 GAT ACC CTA CGA CCA GGA CAA GGC GTG GGG CTG GCT GTC GCG CGC GAG ATT ACG GAA CAA  
 D T L R P G Q G V G L A V A R E I T E Q  
 2765/461 2795/471  
 TAC GCC GGG CAG ATC ATT GCC AGC GAC AGT CTG CTC GGT GGC GCC CGT ATG GAG GTC GTT  
 Y A G Q I I A S D S L L G G A R M E V V  
 2825/481 2855/491  
 TTT GGC CGA CAG CAT CCC ACA CAG AAA GAG GAA TAA  
 F G R Q H P T Q K E E \* ← *phoQ* ends

**Figure 29. Construction of the suicide vector for introducing  $\Delta P_{phoPQ-107::TT}$  araC  $P_{BAD}$   $phoPQ$  deletion-insertion mutation.**

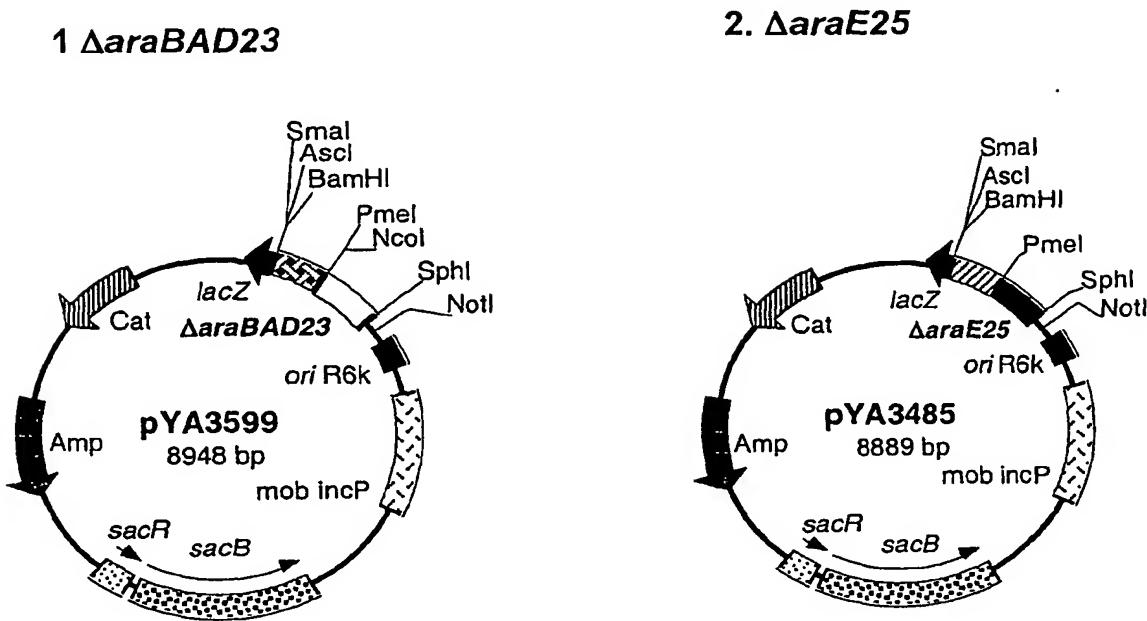


**Figure 30. Chromosomal map of  $\Delta P_{phoPQ-107::TT}\ arac\ P_{BAD}\ phoPQ$  deletion-insertion mutation.**



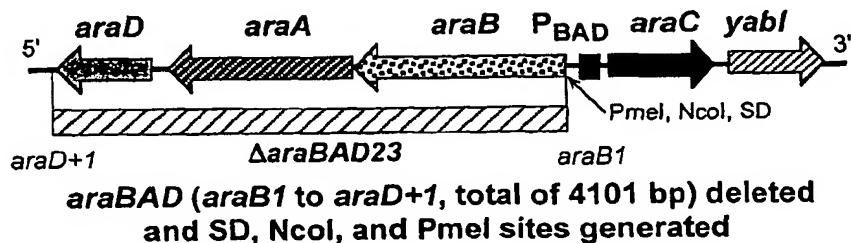
*phoPQ* promoter region (-12 to -109) deleted and 1344 bp  $P_{BAD}$  *araC* TT inserted.

**Figure 31. Diagrams of the suicide vectors for introducing the  $\Delta$ araBAD23 and  $\Delta$ araE25 deletion mutations.**

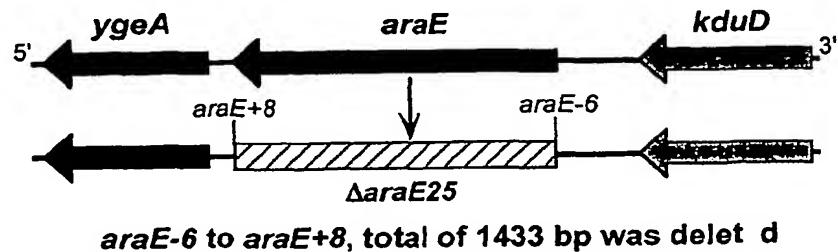


**In *Salmonella* chromosome:**

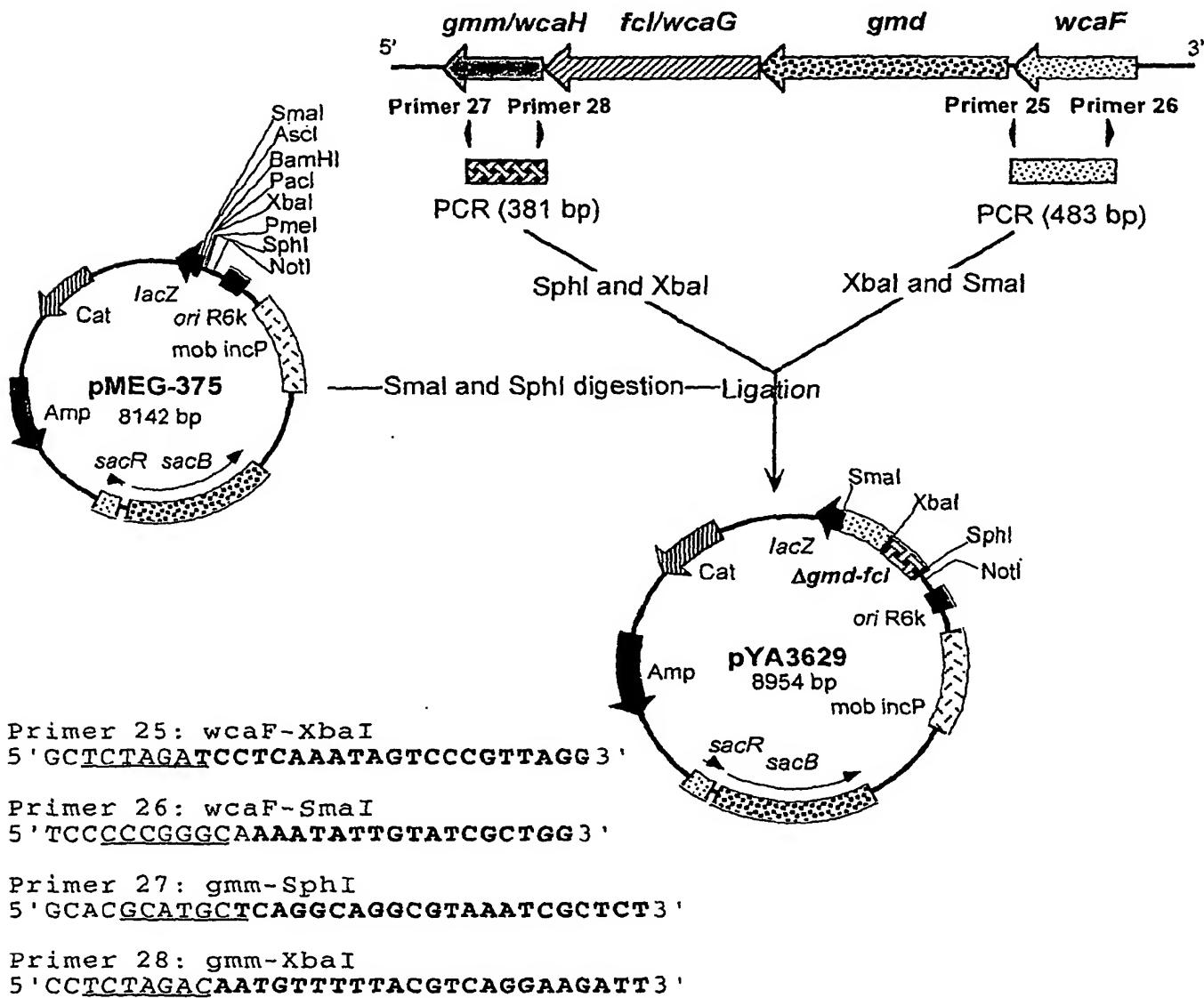
**1.  $\Delta$ araBAD23**



**2.  $\Delta$ araE25**



**Figure 32. Construction of the suicide vector for introducing the  $\Delta(gmd-fcl)$ -26 deletion mutation.**



*gmm/wcaH*: Guanosine di-P mannose mannosyl hydrolase

*fcl/wcaG*: Colanic acid gene cluster, bifunctional GDP fucose synthetase

*gmd*: Fucose biosynthesis; GDP-D-mannose 4,6-dehydratase

*wcaF*: Involved in lipopolysaccharide biosynthesis, putative acyltransferase

**Figure 33. Chromosomal map of the  $\Delta(gmd-fcl)$ -26 deletion mutation.**

**In *Salmonella* chromosome:**

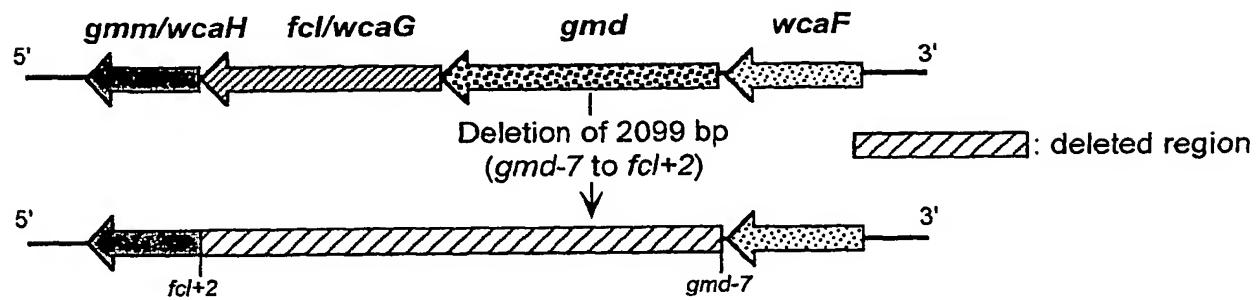


Figure 34. Diagrams of all the suicide vectors listed in Table 2.

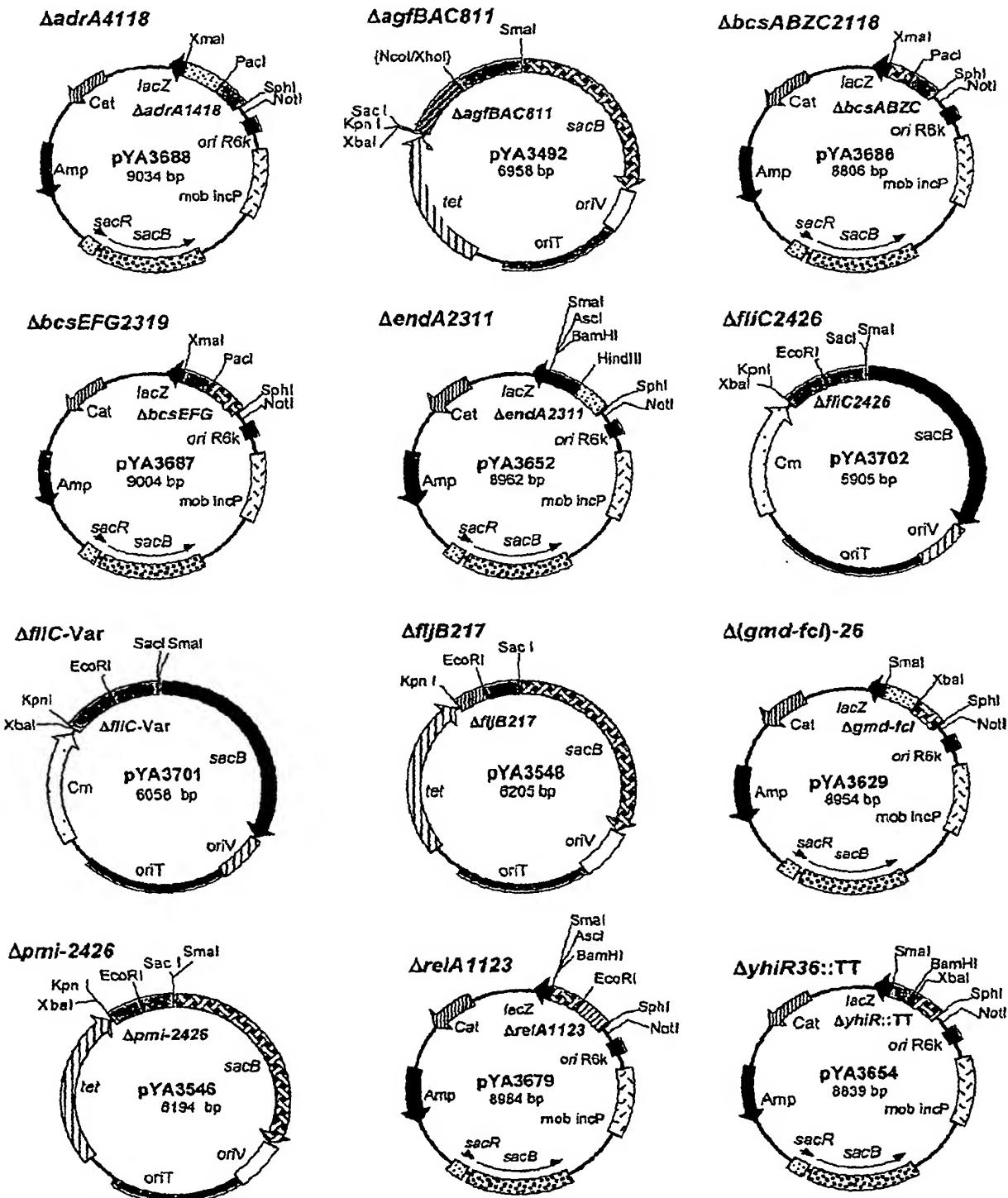
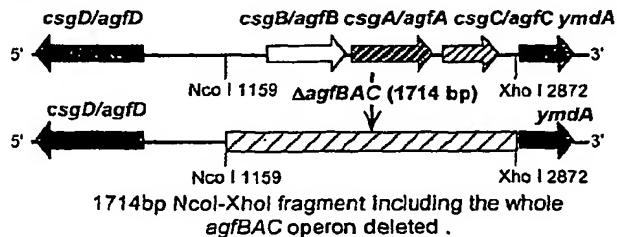
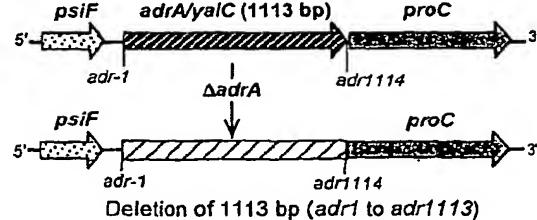
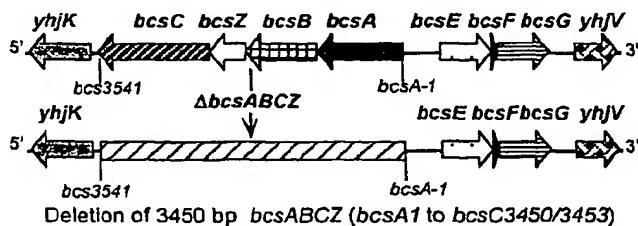
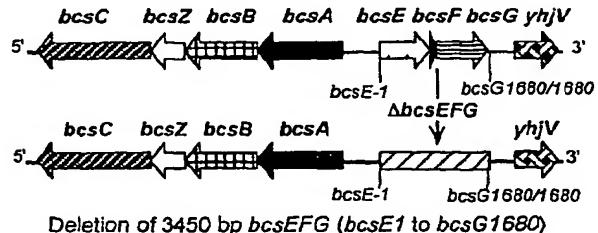
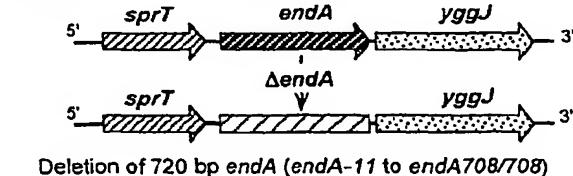
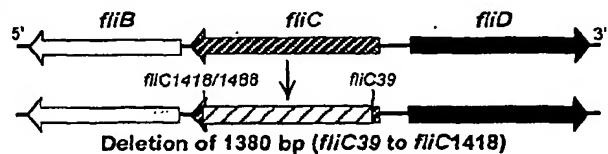
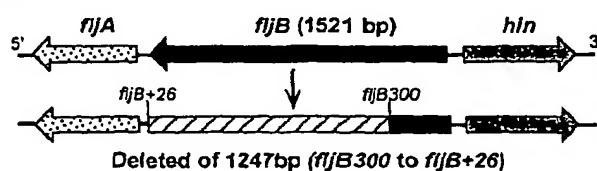
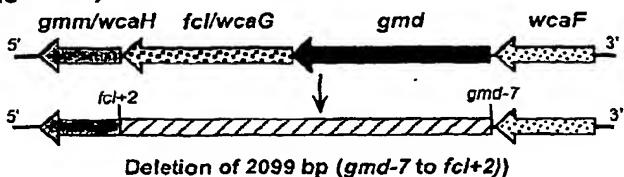
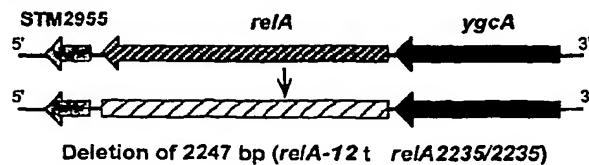
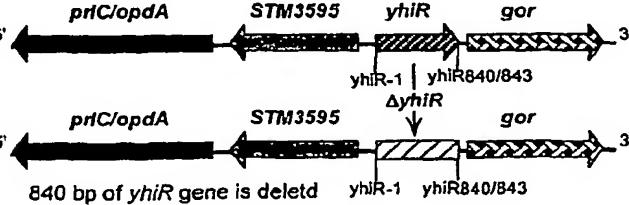


Figure 35. Deletion mutations after insertion into *Salmonella* chromosome. **$\Delta agfBAC811$**  **$\Delta adrA1418$**  **$\Delta bcsABC2118$**  **$\Delta bcsEFG2319$**  **$\Delta endA2311$**  **$\Delta fliC825$**  **$\Delta fliB217$**  **$\Delta(gmd-fcl)-26$**  **$\Delta relA1123$**  **$\Delta yhiR36::TT$** 

████████: deleted region

**Figure 36. DNA and amino acid sequences of *sopB* and the flanking region of *S. typhimurium* chromosome.**

GGA ATA GGA AAA ACG AAT ATT CTT CGT CAC GGT CTT ACT TGT CCG GGG CTT TGC TGG CAT  
 S Y S F R I N K T V T K S T R P K A P M  
 ←STM1092 starts ↑

ACA CAC ACC TGT ATA ACA TTT GAT GTA ACG CCG TTA CTT TAC GCA GGA GTA AAT CGG TGA  
 SD (STM1092)  
 ATT TGA TCT GAG TCA AGA AGG TGG GTT TTC AAT AAA AGT TGT GCC ATA AAT TGT GAA GTT  
 TGT AGA TTT TAT GAA CAT TTG ATG TAC CGA TCT CCC CCA TGA TCG CCA CTA CGT ATG GAC  
 GTC AGG ATG CCT CCC CGC CTG ATC AGA AGC GTT TCC TCA TTA AAA AGG ACA TTT TTT TAA  
 AGT TCC TGG TGC ATA AAA GTC ACA TCC TTT TAA AGG GTT AAC CCT GTT GAA TGT TCC  
 SD  
 CAC TCC CCT ATT CAG GAA TAT TAA AAA CGC T  
 ↑SD-sopB deleted (sopB-18 to sopB1686)

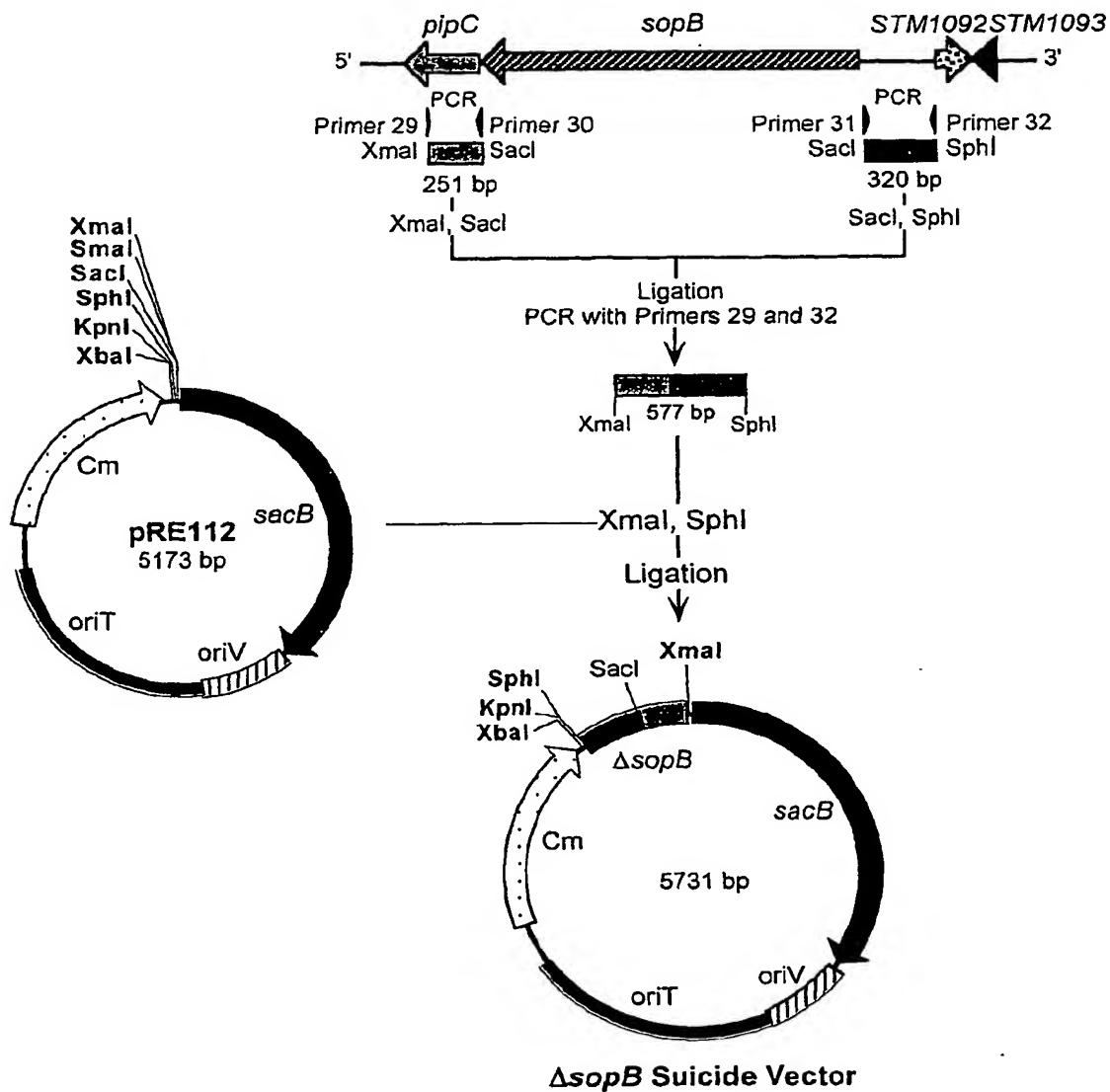
*sopB* starts →

1/1 31/11  
 ATG CAA ATA CAG AGC TTC TAT CAC TCA GCT TCA CTA AAA ACC CAG GAG GCT TTT AAA AGC  
 M Q I Q S F Y H S A S L K T Q E A F K S  
 61/21 91/31  
 CTA CAA AAA ACC TTA TAC AAC GGA ATG CAG ATT CTC TCA GGC CAG GGC AAA GCG CCG GCT  
 L Q K T L Y N G M Q I L S G Q G K A P A  
 121/41 151/51  
 AAA GCG CCC GAC GCT CGC CCG GAA ATT ATT GTC CTG CGA GAA CCC GGC GCG ACA TGG GGG  
 K A P D A R P E I I V L R E P G A T W G  
 181/61 211/71  
 AAT TAT CTA CAG CAT CAG AAG GCG TCT AAC CAC TCG CTG CAT AAC CTC TAT AAC TTA CAG  
 N Y L Q H Q K A S N H S L H N L Y N L Q  
 241/81 271/91  
 CGC GAT CTT CTT ACC GTC GCG GCA ACC GTT CTG GGT AAA CAA GAC CCG GTT CTA ACG TCA  
 R D L L T V A A T V L G K Q D P V L T S  
 301/101 331/111  
 ATG GCA AAC CAA ATG GAG TTA GCC AAA GTT AAA GCG GAC CGG CCA GCA ACA AAA CAA GAA  
 M A N Q M E L A K V K A D R P A T K Q E  
 361/121 391/131  
 GAA GCC GCG GCA AAA GCA TTG AAG AAA AAT CTT ATC GAA CTT ATT GCA GCA CGC ACT CAG  
 E A A A K A L K K N L I E L I A A R T Q  
 421/141 451/151  
 CAG CAG GAT GGC TTA CCT GCA AAA GAA GCT CAT CGC TTT GCG GCA GTA GCG TTT AGA GAT  
 Q Q D G L P A K E A H R F A A V A F R D  
 481/161 511/171  
 GCT CAG GTC AAG CAG CTT AAT AAC CAG CCC TGG CAA ACC ATA AAA AAT ACA CTC ACG CAT  
 A Q V K Q L N N Q P W Q T I K N T L T H  
 541/181 571/191  
 AAC GGG CAT CAC TAT ACC AAC ACG CAG CTC CCT GCA GCA GAG ATG AAA ATC GGC GCA AAA  
 N G H H Y T N T Q L P A A E M K I G A K  
 601/201 631/211  
 GAT ATC TTT CCC AGT GCT TAT GAG GGA AAG GGC GTA TGC AGT TGG GAT ACC AAG AAT ATT  
 D I F P S A Y E G K G V C S W D T K N I

Figure 36. (cont'd.,

661/221 691/231  
 CAT CAC GCC AAT AAT TTG TGG ATG TCC ACG GTG AGT GTG CAT GAG GAC GGT AAA GAT AAA  
 H H A N N L W M S T V S V H E D G K D K  
 721/241 751/251  
 ACG CTT TTT TGC GGG ATA CGT CAT GGC GTG CTT TCC CCC TAT CAT GAA AAA GAT CCG CTT  
 T L F C G I R H G V L S P Y H E K D P L  
 781/261 811/271  
 CTG CGT CAC GTC GGC GCT GAA AAC AAA GCC AAA GAA GTA TTA ACT GCG GCA CTT TTT AGT  
 L R H V G A E N K A K E V L T A A L F S  
 841/281 871/291  
 AAA CCT GAG TTG CTT AAC AAA GCC TTA GCG GGC GAG GCG GTA AGC CTG AAA CTG GTA TCC  
 K P E L L N K A L A G E A V S L K L V S  
 901/301 931/311  
 GTC GGG TTA CTC ACC GCG TCG AAT ATT TTC GGC AAA GAG GGA ACG ATG GTC GAG GAC CAA  
 V G L L T A S N I F G K E G T M V E D Q  
 961/321 991/331  
 ATG CGC GCA TGG CAA TCG TTG ACC CAG CCG GGA AAA ATG ATT CAT TTA AAA ATC CGC AAT  
 M R A W Q S L T Q P G K M I H L K I R N  
 1021/341 1051/351  
 AAA GAT GGC GAT CTA CAG ACG GTA AAA ATA AAA CCG GAC GTC GCC GCA TTT AAT GTG GGT  
 K D G D L Q T V K I K P D V A A F N V G  
 1081/361 1111/371  
 GTT AAT GAG CTG GCG CTC AAG CTC GGC TTT GGC CTT AAG GCA TCG GAT AGC TAT AAT GCC  
 V N E L A L K L G F G L K A S D S Y N A  
 1141/381 1171/391  
 GAG GCG CTA CAT CAG TTA TTA GGC AAT GAT TTA CGC CCT GAA GCC AGA CCA GGT GGC TGG  
 E A L H Q L L G N D L R P E A R P G G W  
 1201/401 1231/411  
 GTT GGC GAA TGG CTG GCG CAA TAC CCG GAT AAT TAT GAG GTC GTC AAT ACA TTA GCG CGC  
 V G E W L A Q Y P D N Y E V V N T L A R  
 1261/421 1291/431  
 CAG ATT AAG GAT ATA TGG AAA AAT AAC CAA CAT CAT AAA GAT GGC GGC GAA CCC TAT AAA  
 Q I K D I W K N N Q H H K D G G E P Y K  
 1321/441 1351/451  
 CTC GCA CAA CGC CTT GCC ATG TTA GCC CAT GAA ATT GAC GCG GTA CCC GCC TGG AAT TGT  
 L A Q R L A M L A H E I D A V P A W N C  
 1381/461 1411/471  
 AAA AGC GGC AAA GAT CGT ACA GGG ATG ATG GAT TCA GAA ATC AAG CGA GAG ATC ATT TCC  
 K S G K D R T G M M D S E I K R E I I S  
 1441/481 1471/491  
 TTA CAT CAG ACC CAT ATG TTA AGT GCG CCT GGT AGT CTT CCG GAT AGC GGT GGA CAG AAA  
 L H Q T H M L S A P G S L P D S G G Q K  
 1501/501 1531/511  
 ATT TTC CAA AAA GTA TTA CTG AAT AGC GGT AAC CTG GAG ATT CAG AAA CAA AAT ACG GGC  
 I F Q K V L L N S G N L E I Q K Q N T G  
 1561/521 1591/531  
 GGG GCG GGA AAC AAA GTA ATG AAA AAT TTA TCG CCA GAG GTG CTC AAT CTT TCC TAT CAA  
 G A G N K V M K N L S P E V L N L S Y Q  
 1621/541 1651/551  
 AAA CGA GTT GGG GAT GAA AAT ATT TGG CAG TCA GTA AAA GGC ATT TCT TCA TTA ATC ACA  
 K R V G D E N I W Q S V K G I S S L I T  
 1681/561  
 TCT TGA GTCTTGAGGTAACTAT ATG GAA AGT CTA TTA AAT CGT TTA TAT GAC GCG TTA GGC  
 S \* ↑ SD M E S L L N R L Y D A L G  
 (*sopB1686*) *pipC* starts

**Figure 37. Construction of the suicide vector for introducing the  $\Delta sopB$  deletion mutation into the *Salmonella* chromosome.**



Primer 29: 5' TTCCCCGGGGCAGTATTGTCTGCGTCAGCG 3' (XmaI-N)  
 Primer 30: 5' TTGAGAGCTCGTCTGAGGTAACCTATGGAAAG 3' (SacI-N)  
 Primer 31: 5' TTGAGAGCTCGAATAGGGGAGTGGGAACATTC 3' (SacI-C)  
 Primer 32: 5' ACATGCAATGCGGCATACACACACCTGTATAACA 3' (SphI-C)

**Figure 38. Chromosomal map of  $\Delta sopB$  deletion mutation.**

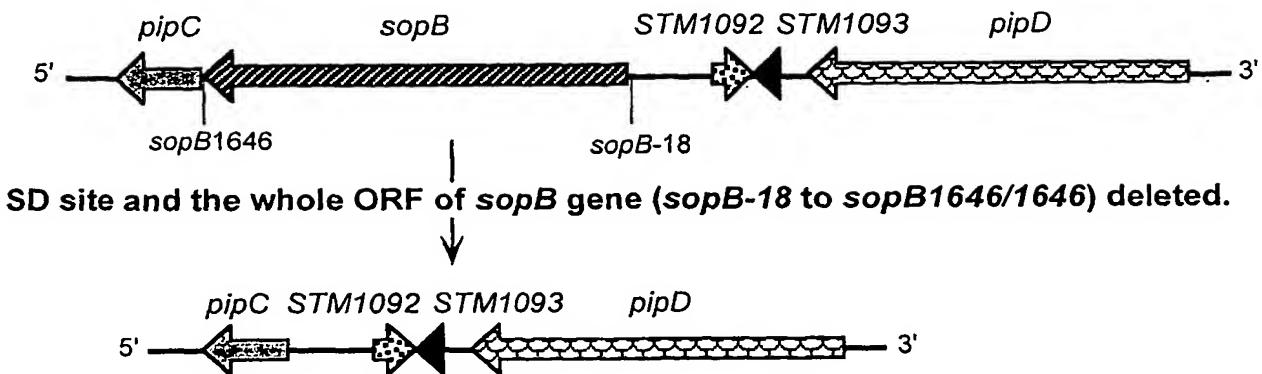
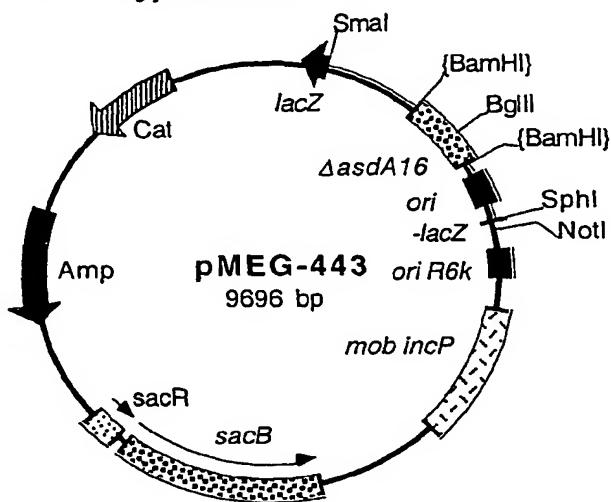
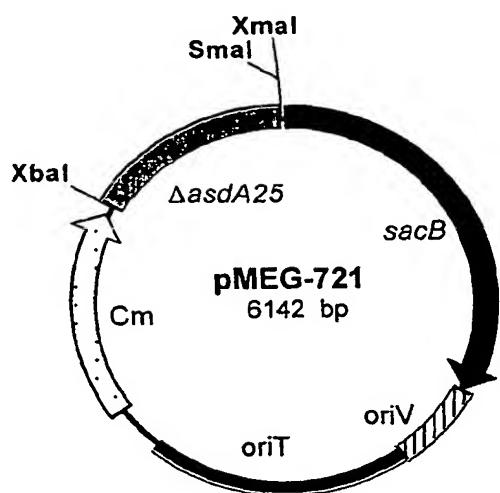


Figure 39. Diagrams of the suicide vectors for introducing  $\Delta asdA16$  into *S. typhimurium* and  $\Delta asdA25$  into *S. paratyphi* A and *S. typhi* strains.

$\Delta asdA16$  for *S. typhimurium*

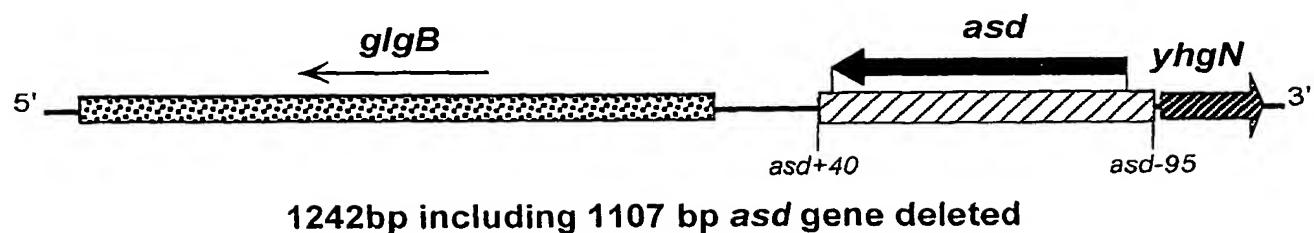


$\Delta asdA25$  for *S. paratyphi* A and *S. typhi*

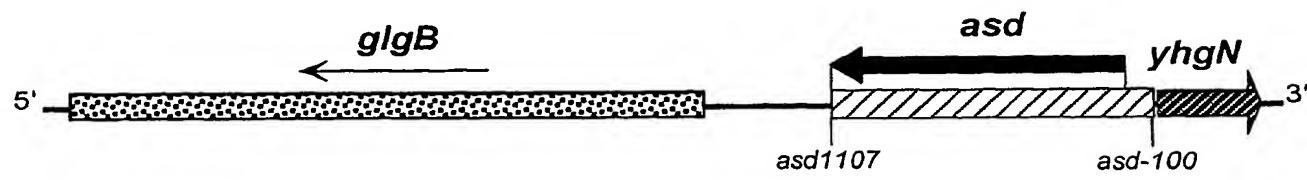


**Figure 40. Chromosomal maps of  $\Delta asdA16$  and  $\Delta asdA25$  deletion mutation.**

**$\Delta asdA16$  for *S. typhimurium***

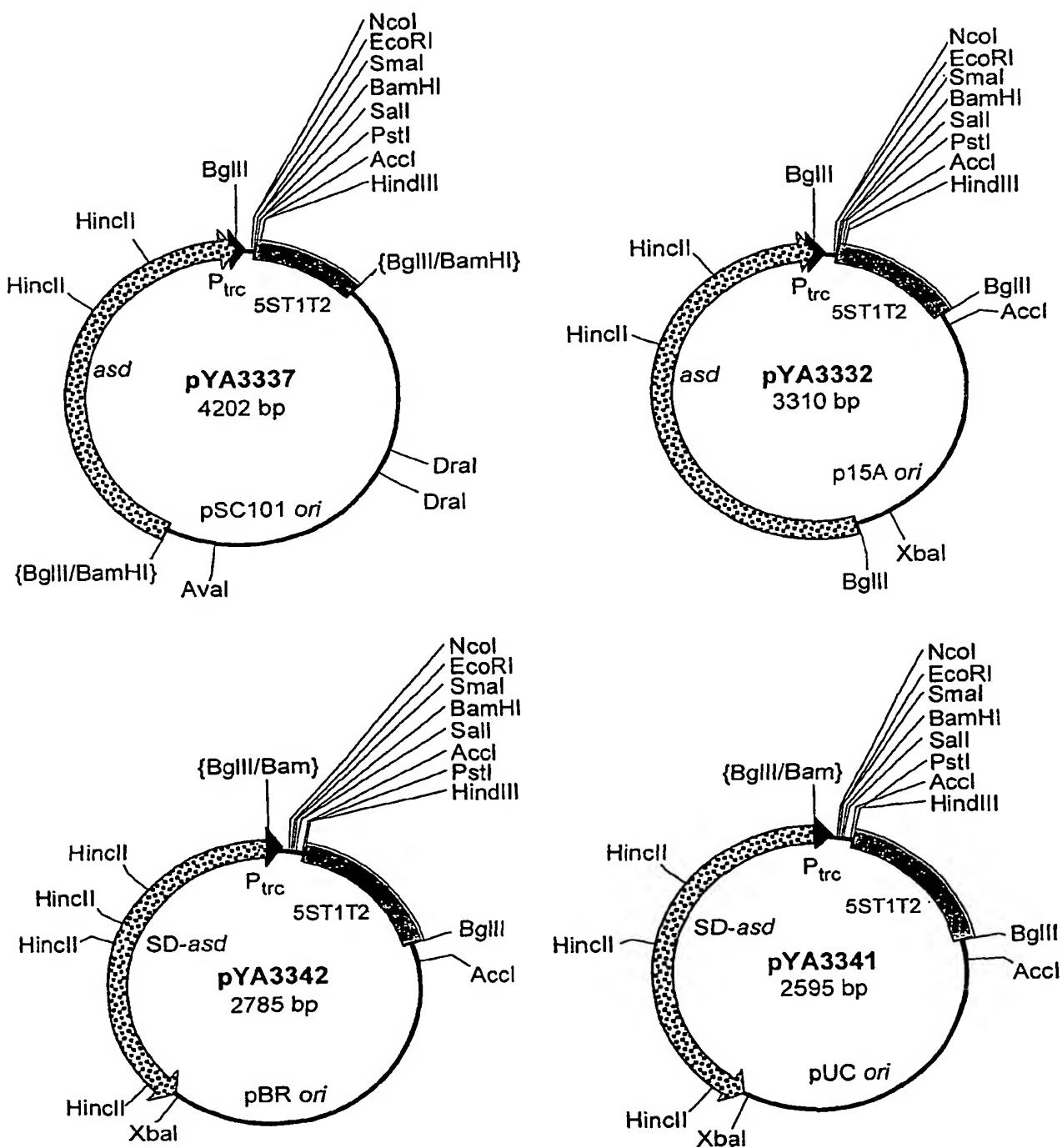


**$\Delta asdA25$  for *S. paratyphi A* and *S. typhi***



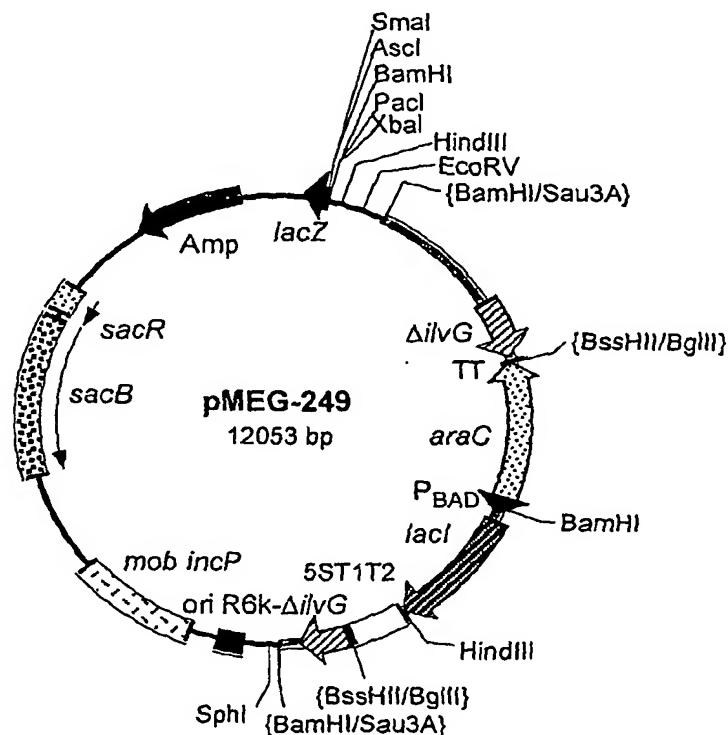
|||||: deleted region

**Figure 41. *Asd*<sup>+</sup> vectors with pSC101, p15A, pBR and pUC origins of replication to regulate plasmid copy numbers.**

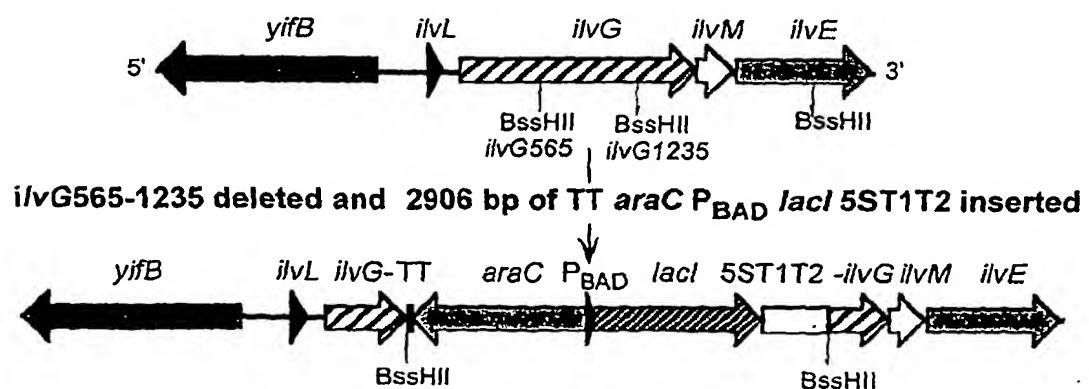


**Figure 42.** Nucleotide sequence of  $P_{trc}$  and the multiple cloning sites (MCS) of  $Asd^+$  vectors in Figure 41.

**Figure 43. Diagram of the suicide vector for introducing  $\Delta ilvG3::TT$  araC  $P_{BAD}$  lacI TT deletion-insertion mutation and map of  $\Delta ilvG3::TT$  araC  $P_{BAD}$  lacI TT mutation in the *Salmonella* chromosome.**



**In *Salmonella* chromosome:**



**Figure 44: Nucleotide and amino acid sequences of *S. typhimurium fimH* and FimH protein**

1/1	31/11
atg aaa ata tac tca gcg cta ttg ctg gcg	ggg acc gcg ctc ttt ttc acc cat ccc gcg
M K I Y S A L L L A	G T A L F F T H P A
61/21	91/31
ctg gcg acc gtt tgc cgt aat tca aac ggg	acg gcg acc gat atc ttt tac gac ctg tca
L A ↑ T V C R N S N G	T A T D I F Y D L S
121/41	151/51
gat gtt ttc acc agc ggc aat aat cag ccg	gga cag gtg gtg acg ctg ccg gaa aaa tca
D V F T S G N N Q P	G Q V V T L P E K S
181/61	211/71
ggg tgg gtc ggc gta aac gcg acg tgc ccg	gcg ggg aca acg gtg aat tat acc tac cga
G W V G V N A T C P	A G T T V N Y T Y R
241/81	271/91
agc tat gta tca gaa tta ccg gta caa agt	acc gaa gga aat ttt aaa tac ctc aag ttg
S Y V S E L P V Q S	T E G N F K Y L K L
301/101	331/111
aat gac tac ctt ctg ggc gcg atg agc atc	acc gat agt gtc gct ggc gta ttt tat ccg
N D Y L L G A M S I	T D S V A G V F Y P
361/121	391/131
ccc cgt aac tat att ctc atg ggc gtc gac	tat aac gtg tcg cag caa aag ccg ttt ggc
P R N Y I L M G V D	Y N V S Q Q K P F G
421/141	451/151
gtg cag gac tca aag ctg gtt ttt aaa tta	aaa gtg ata cgg cct ttt att aat atg gtg
V Q D S K L V F K L	K V I R P F I N M V
481/161	511/171
acg atc cct cgc cag aca atg ttt acc gtc	tat gtg acg acc tct acc ggc gac gcg ttg
T I P R Q T M F T V	Y V T T S T G D A L
541/181	571/191
agc acg ccg gta tat acc att agc tac agc	ggc aaa gtg gaa gtg ccg caa aac tgt gaa
S T P V Y T I S Y S	G K V E V P Q N C E
601/201	631/211
gtg aat gcc gga cag gtc gtg gag ttt gat	ttc ggc gat atc ggc gcg tcg tta ttt agt
V N A G Q V V E F D	F G D I G A S L F S
661/221	691/231
cag gcg ggg gcg ggt aat cgt ccg caa ggc	gtc acg ccg caa acg aaa acc att gct atc
Q A G A G N R P Q G	V T P Q T K T I A I
721/241	751/251
aaa tgt acc aac gtc gcg gcg cag gcc tat	tta tcg atg cgg ctt gaa gcc gaa aag gcc
K C T N V A A Q A Y	L S M R L E A E K A
781/261	811/271
tca ggg cag gcg atg gtg tcc gat aat ccg	gat tta ggc ttt gtg gtt gct aat agc aac
S G Q A M V S D N P	D L G F V V A N S N
841/281	871/291
ggc acg ccg ctt aca ccc aat aat ttg tcg	agt aaa att ccg ttt cat ctt gat gat aac
G T P L T P N N L S	S K I P F H L D D N
901/301	931/311
gcc gcc gct cgc gta ggt att cgc gcc tgg	cca atc agc gtg acg ggg att aaa ccg gcg
A A A R V G I R A W	P I S V T G I K P A
961/321	991/331
gaa ggg ccg ttt act gcg cgc ggc tat cta cga	gtc gat tat gat taa
E G P F T A R G Y L	R V D Y D *

Amino acids 1-22 constitute the signal sequence cleaved from the mature protein (amino acids 23 to 335). Arrow indicates site of signal peptidase cleavage.

Figure 45: Construction of *fimH* *Asd*<sup>+</sup> vectors

Primer1: FimH-NcoI (starting with amino acid 23)

5'- CAT GCC ATG GCA TGC ACG GTT TGC CGT AAT TCA AAC G-3'

Primer2: FimH<sub>100</sub>-HindIII (starting with amino acid 122)

5'-GCC CAA GCT TA TTA ACG GGG CGG ATA AAA TAC GCC AGC-3'

Primer3: FimH-HindIII (starting with terminal codon)

5'- GCC CAA GCT TTT AAT CAT AAT CGA CTC GTA GAT AGC C-3'

